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ORIGINAL ARTICLES

ELECTIVE LOCALIZATION OF BACTERIA FOLLOWING VARIOUS METHODS OF INOCULATION, AND THE PRODUCTION OF NEPHRITIS BY DEVITALIZATION AND INFECTION OF TEETH IN DOGS*

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THERE are many facts which indicate that focal infections play an important part in the etiology of nephritis.[14, 17] Various forms of nephritis have been produced by intravenous injections of streptococci.[15, 16] Improvement, especially in acute and subacute nephritis, occurs often after removal of tonsils and other foci of infection.[3, 6, 8, 11, 13] The tolerance of the kidney to repeated injections of arsphenamine in the treatment of syphilis is greater in persons free from focal infections.[9, 25] The incidence and severity of nephritis and other complications in scarlet fever are far less in persons whose tonsils have been removed than in those in whom tonsillectomy has not been performed.[4, 12] The idea that advanced chronic nephritis is the result of repeated onslaughts and not the result of a continuous process has been recently emphasized.[10]

In the course of numerous experiments in animals lesions of the kidneys were found to occur more often following intravenous injection of streptococci from diseases in which involvement of the kidney occurs than from diseases in which the kidneys are spared.[18] Twenty per cent of the rabbits injected with *Streptococcus viridans* from chronic infectious endocarditis, a disease in which glomerulonephritis due to this organism is commonly

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noted,[2] developed focal lesions in the kidneys. Lesions in the kidneys were noted in 39 per cent of the animals injected with the streptococci from rheumatic fever. The type of lesion in these was such that its repeated occurrence might ultimately result in a scarred contracted kidney, such as develops not infrequently in persons with recurring attacks of rheumatic infections.

During the course of numerous experiments on the intravenous injection of bacteria, especially relatively nonvirulent laboratory strains of streptococci, lesions of the kidney did not occur until after the virulence was increased by animal passage. The affinity for the kidney became greater after the streptococci from appendicitis, ulcer of the stomach, and cholecystitis were passed through a number of animals.[19]

These observations suggest that if focal infections play an important part in the production of nephritis, the foci in these cases may harbor bacteria which have a predilection for the parenchyma of the kidney. This has been found to be the case following the intravenous injection of bacteria from foci of infection in four selected cases of nephritis, representing different types of the disease.[20] Of the thirty-three animals injected with the primary cultures, twenty-seven (82 per cent) developed lesions in the kidneys. The affinity of the streptococci isolated from the lesions in the kidneys of these animals (second animal passage) was also marked, while in the third animal passage it had largely disappeared. The incidence of lesions in other organs was small. The lesions following injection of the strains from chronic nephritis were relatively slight as compared with those following the injection of bacteria from acute nephritis. A selective survival in the parenchyma of the kidney of streptococci having affinity for this organ was also demonstrated. The conditions and type of lesion noted commonly in pyelonephritis have been reproduced in the main by Bumpus and Meisser following intravenous injection of freshly isolated strains of streptococci from dental and other foci of infection in patients with pyelonephritis.

It must not be assumed that it is essential to inject the bacteria intravenously in order that they may localize in the tissues for which they have elective affinity. Characteristic localization has been obtained repeatedly following other methods of inoculation. Marked ulceration of the stomach in guinea pigs occurred following intraperitoneal injection of the streptococcus from a suppurative sinus from an infected tooth in a patient with acute ulcer of the stomach. Suppurative pulpitis and hemorrhagic edema of the periosteum opposite the roots of teeth in animals followed the intraperitoneal injection of the streptococcus from the pulp of a tooth of a patient who had recurring attacks of pulpitis, dental neuritis, and myositis.[21] Localization in the alveolar epithelium of the lungs in guinea pigs occurred after intraperitoneal injection of the highly virulent green-producing streptococcus from influenza.[22] Suppurative endometritis was found often after intratracheal inoculation of this organism, freshly isolated from sputum or lung exudate in cases of severe pulmonary influenza during the epidemic in 1918.[22] Localization of streptococci, with leukocytic infiltration, was found in the ileum following the intratracheal injection of a small amount of hemorrhagic mucus con-

taining streptococci from a patient with intestinal influenza; hemorrhagic colitis developed after the freshly isolated streptococcus, in oil suspension, from another patient with intestinal influenza was injected into the stomach.[23]

The extreme specificity for muscle tissue of a streptococcus from a case of myositis was shown in an experiment in which the organism injected intravenously not only localized in the muscles of a pregnant rabbit, but also in the corresponding muscles of the fetuses.[24] A streptococcus isolated from a tooth of a patient with recurring attacks of iritis, myositis, and arthritis produced iritis, myositis, and arthritis on intravenous injection, and also produced iritis, including the uninjected eye, and lesions in muscles when extremely small doses were inoculated into the anterior chamber of one eye.[20]

In the light of these striking results there is no reason to doubt that specific localization in distant tissues or organs may occur from chronic, localized, often symptomless, infections. It has been shown that the bacteria are in close proximity to the newly forming blood vessels in chronic foci of infection, such as granulomas at the apices of devitalized teeth, and are not incapsulated in scar tissue.[24] The fundamental requirements of the principles involved in the production of lesions remote from a focus of infection have been fulfilled, but in order to test the effect of one type of chronic focus of infection, produced so frequently in dental practice through the devitalization of teeth, teeth in dogs were devitalized and infected with bacteria having elective affinity for various tissues.

THE RESULTS OF EXPERIMENTS WITH A STAPHYLOCOCCUS HAVING ELECTIVE AFFINITY FOR RENAL TISSUE FROM A PATIENT WITH NEPHRITIS: HISTORY OF PATIENT

CASE A 334085. Mr. R. S., aged twenty-one years, came to the Clinic November 8, 1920, with a history of having had repeated attacks of acute nephritis for six years. In a number of these attacks his condition had been critical. Partial recovery had ensued after prolonged rest in bed, a salt-free and protein-low diet, and the usual elimination treatment by sweats and cathartics. In several attacks he had had marked generalized edema, weakness, shortness of breath, headache, nausea and vomiting, and large amounts of albumin and many casts in the urine. On one occasion the urine contained much blood. He had been subject to frequent attacks of "cold in the head" as long as he could remember. Several acute attacks and less severe exacerbations of his renal condition followed nasopharyngeal infections.

Examination revealed a dilated heart and moderate edema of the lower extremities. The systolic blood pressure ranged from 165 to 190, the diastolic from 110 to 140. The hemoglobin was 42 per cent, the erythrocytes 2,460,000. The leukocytes on four occasions were 4,600, 6,400, 2,400 and 15,000. A Wassermann test of the blood, and two blood cultures were negative. The eye grounds were normal. The urine contained a large amount of albumin, and a moderate number of hyaline casts, renal cells, erythrocytes, and leukocytes. The phenolsulphonephthalein test revealed marked impairment of renal function. The blood urea and creatinin were extremely high. A detailed study of the blood chemistry will be reported by Dr. Rowntree, to whom we are indebted for the opportunity of studying this case.

Careful search for foci of infection revealed a cloudy left antrum, inflamed mucous membrane of the nose, especially on the left side, and moderate sized red tonsils from which liquid pus could be expressed. The teeth were normal. Owing largely to the results of the animal experiments reported herein, tonsillectomy was advised and performed, and the

left antrum was irrigated. Following these operations improvement was noted. The attacks of cold in the head were less frequent. Fewer casts, erythrocytes, and leukocytes were found; albumin in the urine became less, the blood urea diminished, and a moderate degree of health and strength returned. The patient's complete recovery appears out of the question because of the long duration of the disease and the probable extensive destruction of the kidney.

Cultures from the mucous membrane of the nose and from the washings of the left antrum yielded countless numbers of staphylococci in almost pure culture, while cultures from the emulsion of the tonsils yielded an unusually large number of staphylococci, together with hemolytic and green-producing streptococci. The effect of intravenous injection of the primary culture in glucose-brain broth (3 to 5 c.c. for adult rabbits) was studied in all in thirty-two rabbits. The affinity of the staphylococcus for the kidneys was marked. The streptococci failed to localize and were lost in the first animal passage. The percentage incidence of lesions which occurred in the various organs, in the first and subsequent animal passages, may be seen in Table I.

TABLE I

	ANIMALS INJECTED	PERCENTAGE INCIDENCE OF LESIONS IN						
		KIDNEYS	BLADDER	MUSCLES	JOINTS	MYOCARDIUM	PERICARDIUM	STOMACH
When isolated	11	82	9			18		
First, second, and third animal passages	11	100		27	55	27	9	9
Fourth, fifth, and sixth animal passages	10	100		40	10	80	20	20

Of the eleven rabbits injected with the primary cultures directly from the patient, six were injected with cultures obtained from the left antrum, and the mucous membrane of the nose, three with cultures obtained from the tonsils, and two with a suspension, in salt solution, of pus expressed from the tonsils; all except these two developed marked lesions in the kidneys. The incidence of lesions in other organs in the first animal passage was relatively slight. On successive animal passage, however, the incidence of lesions in other organs increased even though the organism retained its specificity for kidney tissue. A catheterized specimen of urine obtained before injection from each of the thirty-two rabbits was found to be normal in twenty-nine. Of the three remaining, one showed a trace of albumin, one a trace of albumin with a few pus cells and erythrocytes, and the other a moderate amount of albumin and a moderate number of erythrocytes in the urine. The bladders of twenty-five rabbits at necropsy contained sufficient urine for examination. In one of these the urine was normal, in twenty it contained albumin, a large amount in four instances, a moderate amount in sixteen, in four only a trace was present. Pus cells were found in the sediment in twenty-four, relatively few in twenty-one, a larger number in three. Usually there were no casts or erythrocytes, and if they occurred, were few in number.

The staphylococcus produced a yellowish growth on blood-agar and Loeffler's medium. Morphologically it resembled *Staphylococcus aureus* of furunculosis, but its virulence was relatively low. Of the thirty-two rabbits

injected fourteen died in from one to seven days. The others were chloroformed for examination in from three to seven days after injection.

On microscopic examination of sections of the kidneys of the rabbits injected intravenously large areas of focal necrosis, chiefly in the medulla, surrounded by dilatated vessels, were the striking picture, although sometimes small hemorrhages with necrosis of cells and leukocytic infiltration were marked features. Numerous small areas of slight leukocytic and round-cell infiltration were found between the tubules in the cortex. Besides these sharply localized lesions larger areas were found, often involving one or more medullary rays, in which there were marked necrosis of interstitial capillary endothelium, leukocytic infiltration between tubules, and swelling and nonstaining of nuclei of tubular epithelium (Fig. 1). In the medulla corresponding to these areas the tubules, especially Henle's ascending limb, were often

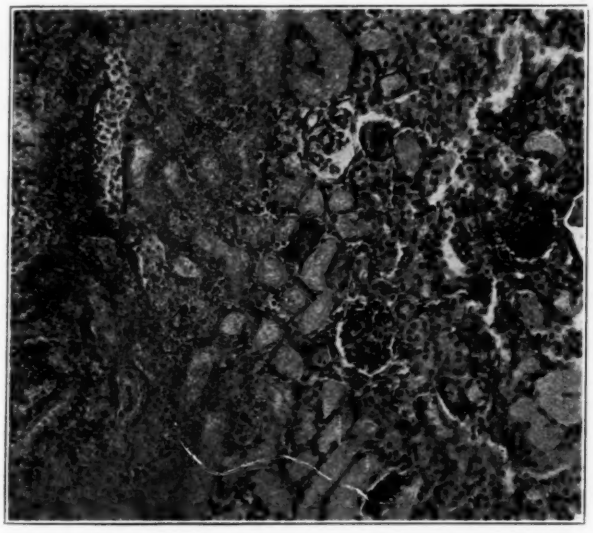

Fig. 1.

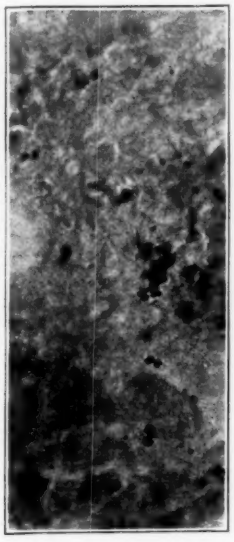

Fig. 2.

Fig. 1.—Section of kidney of rabbit (Rabbit 391) injected intravenously five days previously with the staphylococcus. Area of marked interstitial infiltration (left), adjacent area of swelling and nonstaining of nuclei of tubular epithelium, and the area of normal kidney parenchyma (right). Hematoxylin and eosin (X 100).

Fig. 2.—Staphylococci in area of intense interstitial infiltration shown in Fig. 1. Gram-Weigert (X 1000).

filled with varying proportions of degenerated and desquamated epithelium and leukocytes. The glomeruli were usually spared except as secondarily involved from intense infiltration of surrounding tissue. Bacteria were readily demonstrable (Fig. 2) in the different lesions within forty-eight hours after injection; after that the demonstration of bacteria was difficult, and sometimes it was not accomplished in the areas of diffuse infiltration.

About four months and about six months after the removal of the tonsils and irrigation of the antrum the staphylococcus was again isolated from the nasal mucous membrane and again produced lesions in the kidneys of rabbits on intravenous injection, but these were relatively slight. The affinity

for renal tissue of this organism, isolated from the tonsils and nasal mucous membrane in the first series of experiments, and the lesions on intravenous injection, were so marked that it was thought to be a favorable strain with which to produce nephritis through a chronic focus of infection.

Pure cultures in glucose-brain broth of the staphylococcus recovered from lesions in the kidneys of the rabbits were used to infect the pulp chambers of teeth in dogs. Active, well nourished, vigorous animals were selected. Catheterized specimens of urine from all were examined and found to be normal. In devitalizing and infecting the teeth the animals were covered with a sterile sheet, and kept under ether anesthesia by the intratracheal method. A rubber dam was applied to the mouth, isolating the teeth to be worked on. The teeth were scrubbed, and sterilized with alcohol and tincture of iodine, then cut off with sterile bone nippers midway between the incisal edge and the gum margin. The pulps of the two lower cuspids were carefully removed with sterile broaches, and after the hemorrhage had ceased the bacteria, in dense suspension, were introduced into the pulp chambers with a fine capillary glass pipette. The canal was then sealed with an impervious dental cement. The animals were numbered, kept under hygienic conditions, and fed a balanced diet of dog biscuit, with the occasional addition of meat, as were the control dogs. Catheterized specimens of urine were obtained and examined at intervals. Cultures, and gross and microscopic examinations were made of one kidney, which was removed by Dr. C. S. Williamson in from one to two months after the teeth were infected, so that the findings could be compared with those of the opposite kidney examined after a longer duration of the experiment.

Cultures from the kidney were made by inoculating blood-agar plates and glucose-brain broth with pipetted material after searing the surface of the kidney, and by placing partially macerated pieces of the kidney in glucose-brain broth. The localizing power of the bacteria isolated from the focus and from the lesions in the kidney was tested by intravenous injection into rabbits. Portions of the kidney showing lesions were fixed in 10 per cent formalin, and sections were mounted in paraffin and stained with hematoxylin and eosin to determine the character of the lesions, and by the Gram-Weigert method for the demonstration of organisms.

December 10, 1920, the pulps of the two lower cuspids were removed from Dog 2, and the pulp chambers infected with the staphylococcus from the lesions in the kidney of one of the rabbits injected with the culture from the washings of the left antrum of the patient. There was no swelling or tenderness of the dog's jaw following the devitalization.

January 5, 1921, the animal appeared well. A catheterized specimen of urine contained leukocytes, a few erythrocytes, a trace of albumin, and calcium oxalate crystals. The right kidney, which was removed, appeared normal on the surface, and the capsule stripped readily. The cortex was normal. In the medulla a number of small radiating elongated areas of marked hyperemia were found. Cultures of this kidney remained sterile. Microscopic examination revealed a number of small areas of hemorrhage with slight leu-

kocytic infiltration in the cortex, a number of small areas of necrosis in the medulla, with little or no cellular infiltration, and one larger area of focal necrosis in the medulla with marked leukocytic and round-cell infiltration and degeneration of the tubular epithelium. A few staphylococci were found in this area; in the smaller areas of necrosis and hemorrhage, bacteria were not demonstrated. February 12, the dog died and was examined within an hour. It was emaciated. The devitalized teeth were markedly discolored, but firm, and the cement was in proper place. There was no purulent exudate in the nose. Examination of the lungs revealed small areas of bronchopneumonia and several small abscesses filled with yellowish pus. Smears of the pus contained large numbers of staphylococci. The pleural cavities were empty, and the pleura was smooth. There were no lesions in the stomach, liver, gall

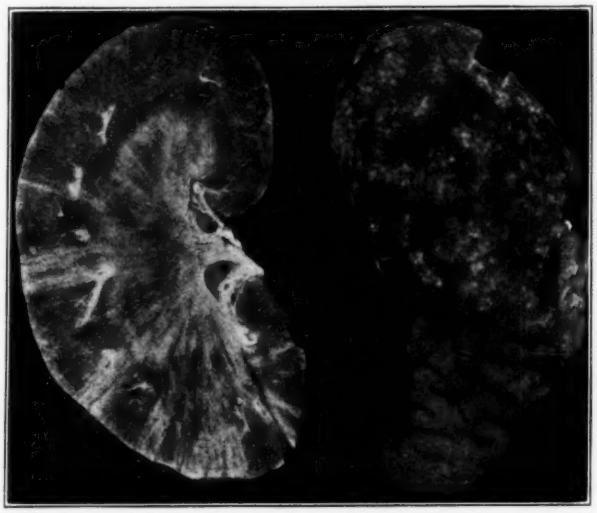

Fig. 3.—Kidney of Dog 2 showing widely disseminated areas of focal nephritis.

bladder, urinary bladder, muscles, heart, joints, or nerve trunks. The remaining kidney was swollen. Numerous whitish areas were seen through the capsule, particularly in the upper two-thirds. The capsule was adherent opposite a number of these areas, but otherwise stripped readily. The cut surface of both the cortex and the medulla presented large and small grayish white, irregularly shaped areas, and streaks with intervening portions of more normal renal tissue (Fig. 3). Some of these areas conformed to the size and shape of medullary rays and appeared almost necrotic, but in none had softening occurred.

On microscopic examination it was found that the larger whitish areas, especially in the cortex, consisted of intense interstitial endothelial hyperplasia and infiltration, with large cells, probably fibroblasts, small round cells, and plasma cells in variable proportions. The large mononuclear cells predominated over wide areas and in these many karyokinetic figures were found (Figs. 4 and 5). Some areas were clearly embolic in origin, the center being necrotic; the structure of the parenchyma was lost. In other areas no

central necrosis was found, but there was instead a more diffuse, widely disseminated infiltration in which the structure of the kidney was obscured. The infiltration often completely or partially surrounded blood vessels and malpighian corpuscles, but the capillaries in the glomeruli and the epithelium of Bowman's capsule were normal (Fig. 4) except that in some instances the cellular infiltration extended along the vessels as they pierced the capsule. In some areas connective tissue formation was marked (Fig. 6). Numerous small areas of interstitial infiltration were found, especially in the medulla, where the collecting tubules and the ascending limb of Henle's loop were often filled with degenerated, desquamated epithelial and other cells. These were usually found in direct line with the larger wedge-shaped areas of cellular infiltration in or near the cortex. The areas varied in size from that of an accumulation of a few leukocytes in the capillaries or tissue spaces between the tubules to

Fig. 4.—Section of kidney of Dog 2. Diffuse proliferation and infiltration by round cells, and beginning formation of interstitial connective tissue; glomerulus normal. Hematoxylin and eosin (X 50).

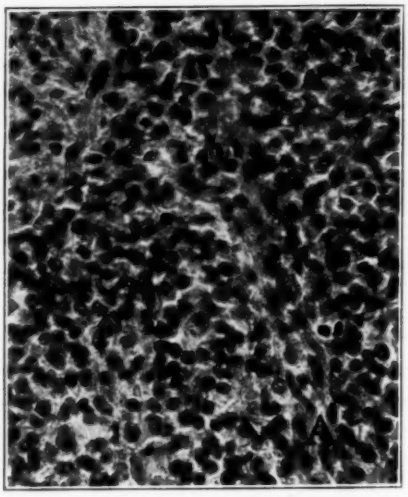

Fig 5.—Higher magnification of area in small circle in Fig. 4, showing character of round cells and karyokinetic figures (*a*). Hematoxylin and eosin (X 500).

large areas in which many tubules were involved. In the more normal kidney structure only occasional areas of hemorrhage, necrosis, and infiltration were found. Prolonged search was necessary to demonstrate the presence of the staphylococcus in the lesions. No other bacteria were found.

Cultures from the kidney and the urine in glucose-brain broth yielded a pure growth of the staphylococcus. Those from the blood, bile, and spleen were negative. Cultures from the lung abscesses yielded staphylococci, streptococci, and *Bacillus bronchisepticus.*

A roentgenogram of the dog's lower jaw showed evidence of periapical rarefaction of the two devitalized cuspids (Fig. 7). On removing the alveolar process over the apex of each tooth a rarefied area 3 to 5 mm. in diameter was

broken into. The tissues were soft and edematous, and the pulp chambers were filled with a dark brown, thick fluid, in which large numbers of gram-positive cocci, often in clumps, streptococci, and small gram-negative bacilli and leukocytes were found. Blood-agar-plate cultures made from these areas showed staphylococci, green-producing streptococci and some gram-positive bacilli.

The localizing power of the staphylococcus isolated from the urine and kidney of this dog was determined by intravenous injection into rabbits. The two injected with the culture from the urine developed marked lesions of the kidney, and one slight lesions of muscles. One of the three injected with the kidney culture developed marked lesions in the kidney; the other two, slight lesions. The staphylococcus was isolated in pure culture from the kidney

Fig. 6.—Section of kidney of Dog 2. Area of well advanced connective tissue formation surrounded by intense interstitial round-cell infiltration. Hematoxylin and eosin (X 100).

Fig. 7.—Roentgenogram of lower jaw of Dog 2, showing large rarefied areas at the apices of the two lower cuspids, which were devitalized and infected.

lesions in all of the rabbits. It was thought worth while to see whether the highly specific localizing power could be made to disappear by injecting the strain into a relatively insusceptible animal. Two guinea pigs were injected intraperitoneally. Both remained well, and were anesthetized for examination seventeen days after injection. An abscess containing thick pus was found in the peritoneum of each. Cultures of the pus yielded a pure growth of the staphylococcus. Two rabbits were injected intravenously with the primary culture in glucose-brain broth. Both remained well and were anesthetized in forty-eight hours. No lesions were found in the kidneys or other organs, and the strain was lost.

December 16, 1920, the pulps were removed from the lower cuspids of

Dog 3, and the pulp chambers infected with the staphylococcus recovered from the lesions in the kidney of a rabbit injected with the cultures from the patient's tonsils.

January 4, 1921, the animal appeared well. A catheterized specimen of urine showed erythrocytes and leukocytes, but no albumin or casts. February 19, the animal had lost in weight. The right kidney was removed. The capsule was adherent opposite eight small whitish scars which on section appeared to be healed embolic areas of focal necrosis. Microscopic examination showed small areas of interstitial, round-cell infiltration between the tubules in the cortex, and in the glomeruli and immediately adjacent to them. The medulla was free from lesions. March 4, the animal was found dead. It had lost much in weight. The devitalized teeth were markedly discolored,

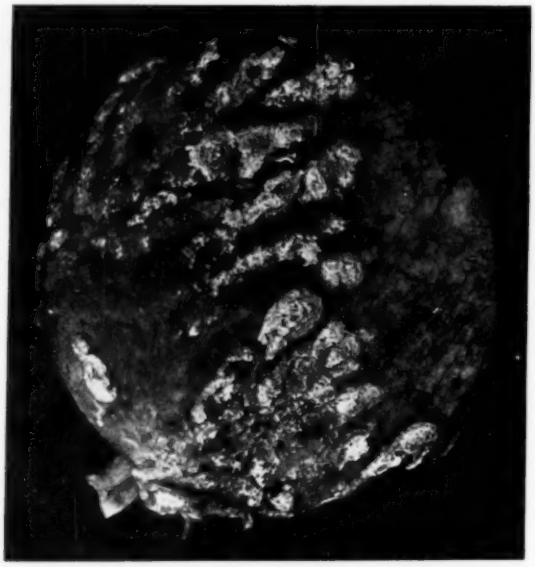

Fig. 8.—Bladder of Dog 6, showing calcareous deposit in adherent exudate.

but firm. The lungs contained a few atelectatic areas and a few patches of peribronchial consolidation. The stomach had a moderate number of hemorrhages in the submucosa. The remaining kidney was enlarged. The capsule stripped readily, and beneath it were found six small whitish areas of necrosis in various stages of healing. By inserting a pipette into the pelvis a large amount of thick grayish pus was obtained. The pelvis was filled with pus and a thick adherent membrane, in which numerous concretions were found. The mucous membrane was edematous and hemorrhagic. The cortex was swollen and grayish in color. The cortical markings were distinct. In the medulla there were a moderate number of linear grayish yellow streaks, surrounded by a hyperemic zone.

The left ureter was hyperemic and swollen, especially in the upper 3 cm., where numerous linear and punctate hemorrhages and an adherent membranous exudate were found. The right ureter also contained punctate

hemorrhages in the mucous membrane, but there was no evidence of infection where it had been severed from the kidney some weeks before.

The wall of the bladder was edematous throughout. The mucous membrane was hyperemic and had many areas of hemorrhage. Adherent to the mucous membrane were many nonlaminated, long, rough, pumice-like concretions which were grayish yellow and which radiated in a fan-like manner from the base of the bladder up along the blood vessels (Fig. 8). On chemical analysis these calcareous deposits were found to contain calcium oxalate and calcium phosphate. The urine contained many leukocytes and erythrocytes, and a large amount of albumin.

Sections of the kidney showed marked swelling of the epithelium of the convoluted tubules, and small areas of hemorrhage, necrosis, and infiltration in the medulla, and a few gram-positive cocci.

Smears of the pus from the kidney contained enormous numbers of gram-positive cocci, often in clumps, large gram-positive and gram-negative bacilli, and a few chains of gram-positive cocci. Cultures of the pus in the pelvis, of the urine, and from the devitalized teeth, yielded large numbers of staphylococci, gram-positive and gram-negative bacilli, and streptococci. The cultures of the blood and of the substance of the kidney remote from the pelvis yielded a pure growth of staphylococci.

One of the two rabbits injected intravenously with the staphylococcus from the parenchyma of the kidney developed lesions in the kidney, the other, abscesses in muscles. Both of the rabbits injected with the staphylococcus from the pus in the pelvis developed lesions in the kidneys. The two injected with the gram-positive bacillus from one infected tooth remained well and no lesions were found at necropsy.

December 30, 1920, the pulp was removed from the two lower cuspids of Dog 5, and the pulp chambers were infected with the staphylococcus recovered from the lesions in the kidney of a rabbit injected with cultures obtained from the mucous membrane of the nose of the patient.

January 4, 1921, a catheterized specimen of urine contained erythrocytes and leukocytes. March 26, the right kidney was removed, and four distinctly opaque, adherent, but healed, thickened areas of scar tissue in the cortex beneath the capsule were found. Beneath several of these were wedge-shaped scars running well into the substance of the cortex and of the medulla. The cortex was cloudy and distinctly swollen. In the medulla were found wedge-shaped areas of marked hyperemia and numerous very small whitish streaks radiating outward from the pelvis and papilla. Cultures made from the kidney yielded staphylococci. Microscopic examination of sections of the kidney revealed the fact that the small whitish streaks found in the medulla were due to interstitial infiltration by small and large round cells in which mitotic figures were easily demonstrable. In the cortex were numerous very small areas of interstitial infiltration, frequently surrounding malpighian corpuscles, with at times invasion of glomeruli along the blood vessels of the hilum.

April 20, the urine contained leukocytes and a trace of albumin. The

animal had lost much in weight and much of its hair, and had developed a corneal ulcer in each eye. April 25, it was anesthetized and examined for lesions. The remaining kidney had numerous very small grayish white streaks radiating from the papilla outward. These lesions were not so marked or so numerous as in the kidney removed at operation.

Cultures made from the urine yielded only staphylococci, from the kidney many staphylococci and an occasional short chained streptococcus. From the apex of the devitalized teeth many staphylococci and an occasional short chain of streptococci were recovered. Cultures made from the fluid in the anterior chamber of the dog's eyes yielded staphylococci and a few streptococci. Cultures from the other organs were negative.

Fig. 9.

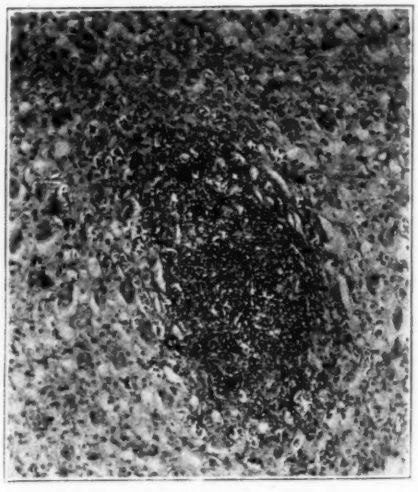

Fig. 10.

Fig. 9.—Photomicrograph of granuloma from apex of devitalized and infected tooth of Dog 5. Note the variations in the intensity of cellular infiltration in the connective tissue, and invasion of the bone. Hematoxylin and eosin (X 50).

Fig. 10.—Photomicrograph of focal lesion in the medulla of kidney of Dog 6. Hematoxylin and eosin (X 70).

Sections of the kidney showed a few small circumscribed areas of round-cell infiltration in the interstitial tissue of the medulla, and larger areas of highly cellular, newly formed connective tissue surrounding the larger blood vessels in the cortex. Bacteria were not found.

Sections of the granuloma at the apex of one of the teeth showed dense connective tissue in which numerous sharply circumscribed areas of intense and mild cellular infiltration were found (Fig. 9). These areas were most numerous at the periphery of the granuloma at the point of erosion of the bone, but were also found in the denser areas of leukocytic infiltration immediately adjacent to the newly forming blood vessels.

December 12, 1920, the two lower cuspids of Dog 6 were devitalized and the pulp chambers infected with a pure culture of the staphylococcus obtained from the lesions in the kidney of a rabbit injected with the culture from the patient's tonsils.

January 4, 1921, the animal appeared well. A catheterized specimen of urine contained erythrocytes and leukocytes, and a trace of albumin. February 19, the right kidney was removed. The capsule stripped readily, and there was no evidence of lesions in the cortex. On the cut surface, with the aid of a magnifying glass, numerous exceedingly fine linear grayish yellow streaks were found in the medulla. These were most numerous in the papillae and appeared on cross section as grayish yellow punctate areas. Cultures made from the medulla by means of a sterile pipette yielded a pure culture of staphylococci. Sections showed a moderate number of small areas of necrosis in the medulla, with little or no cellular infiltration, and numerous small

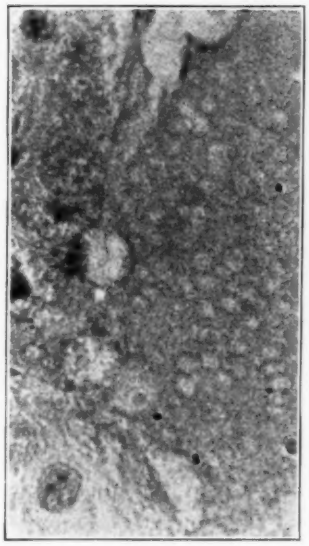

Fig. 11.—Cocci in area of hemorrhagic edema in cortex of kidney of Dog 6. Gram-Weigert (X 1000).

collections of round cells between tubules higher up in the medulla and in the cortex, and adjacent to the malpighian corpuscles. There was moderate congestion of the capillaries and the glomeruli, and small areas of hemorrhage between the tubules in which leukocytes were found. Bacteria were not demonstrated. March 9, the dog had lost much in weight, and appeared sick; it was chloroformed for examination. In the remaining kidney were numerous exceedingly fine linear streaks throughout the medulla. On microscopic examination of sections these were found to consist of aggregations of leukocytes within capillaries and tissue spaces between the tubules, collections of leukocytes and desquamated degenerated epithelial cells in the collecting tubules and ascending limb of Henle's loop. A number of large areas of necrosis with marked infiltration were found in the distal portion of the medulla (Fig. 10). Small areas of interstitial hemorrhage and edema were

found in the cortex, and small infiltrations of leukocytes and round cells in the small vessels between the tubules. In these, staphylococci only (Fig. 11), usually singly, but occasionally in clumps, often within the capillaries, were found, whereas, in the larger areas of necrosis in the medulla staphylococci and streptococci were easily demonstrable.

Cultures of the substance of the kidney and of the urine yielded staphylococci and green-producing streptococci, those from the infected material from the devitalized teeth, the same organisms, together with gram-positive and gram-negative bacilli.

A roentgenogram of the lower jaw showed large areas of rarefaction around the roots of both cuspids. The teeth were firm, and the cement firmly in place. The pulp chambers were moist with chocolate-colored fluid; the tissues over the apices were edematous and the bone was spongy; a well formed granuloma, about 4 mm. in diameter, was found over the root apex of the right cuspid, but none was found over the left cuspid.

Pure cultures of the organisms isolated from the kidney and from the tooth were injected intravenously into rabbits. Two rabbits were injected with the staphylococcus recovered from the kidney. Both developed lesions in the kidneys. Four rabbits were injected intravenously with the green-producing streptococcus from the kidney. All remained well, and the organs were free from lesions. One rabbit was injected with a culture of staphylococcus obtained from the granuloma and apex of the tooth. This animal had lesions in the kidneys. A mixed culture of gram-positive bacilli and green-producing streptococci and staphylococci (gram-positive bacillus was the predominating organism) was injected into four rabbits. One developed slight lesions in the kidney, myocardium, and muscles; the other three were free from lesions.

DISCUSSION AND SUMMARY

Neither tenderness nor swelling of the jaw followed the devitalization of teeth. The devitalized teeth were firm, but markedly discolored. Evidence of infection in the periapical region of each was found at necropsy. In three quite firm masses of moist connective tissue resembling granulomas were found. In the others the softening and infiltration of the bony structure was more diffuse and connective tissue formation was less marked. The pulp chambers usually contained dark, turbid fluid, in which were many bacteria and but few leukocytes. The staphylococcus was recovered from the devitalized teeth in each dog at the end of the experiment, and the elective affinity for kidney tissue, as shown by intravenous injection into rabbits, was still present in the cultures from the two dogs in which this point was tested. In some of the devitalized teeth secondary infection by streptococci and bacilli had taken place. These organisms did not manifest a predilection for kidney tissue on intravenous injection into rabbits.

The etiologic relationship of the staphylococcus inoculated into the teeth to the lesions in the kidneys in these experiments seems established. It was isolated from both the focus and the kidneys. It manifested elective localizing power for the kidneys of rabbits when injected intravenously. It was demon-

strated in the lesions and was not demonstrable in the normal portions of the substance of the kidney. The lesions in three of the dogs were progressive; in one dog healing was well advanced. Lesions in other organs were slight or absent. In two dogs (Dogs 3 and 6) secondary infection of the lesions in the kidneys occurred. In the former, which showed marked pyelitis and cystitis, gram-positive and gram-negative bacilli, streptococci, and staphylococci were found. In the latter, which had interstitial lesions, especially in the medulla, streptococci and staphylococci were demonstrable. Lesions in the parenchyma of the kidney occurred in all four dogs. In three there were no lesions in the mucous membrane lining the pelvis, ureter, and bladder; in one (Dog 3) there were marked pyelitis and cystitis. The finding of marked calcareous deposits in the adherent exudate in the bladder of this dog emphasizes the importance of infection as a factor in the causation of urinary calculi.

Lesions in the kidneys of the character obtained in this experiment did not occur in seven dogs in which the corresponding teeth were devitalized and infected with organisms from sources in patients other than those with nephritis, or in dogs kept under the same conditions but in which the teeth were not devitalized.

The staphylococcus in this experiment was peculiar in that it had such marked affinity for the kidney on intravenous injection, greater than that found in other strains of staphylococcus. This property tended to disappear on successive animal passage on intravenous injection and was entirely lost in the pus in abscesses in the guinea pig following intraperitoneal injection, but was retained for months in the infected teeth and kidneys of the dogs.

The lesions were, in the main, focal in character, although large, widely disseminated areas were found, especially following the devitalization and infection of the teeth of the dogs. In these areas there were marked diffuse interstitial proliferation and infiltration by round cells, and in some marked connective tissue formation besides.

The defensive mechanism of the kidney against bacterial invasion has been found to be marked and to be chiefly cellular in character. Extensive interstitial infiltration not by leukocytes, but by large and small round cells, occurred in response to localization of the staphylococcus. Indeed in some instances (Dog 2) the microscopic picture resembled very closely the findings recorded as characteristic of acute interstitial nephritis.[1, 7] The demonstration of the staphylococcus in these diffuse areas of infiltration was difficult, and yet the proliferation must have been incited by this organism since all gradations occurred between the more strictly focal lesions in which the bacteria were easily demonstrable and the diffuse areas. This finding suggests that the inability to demonstrate bacteria in certain forms of nephritis does not mean that they may not be the inciting agent in some instances. Striking as these findings in the kidneys were, the general picture in the dogs was quite different from that in the patient. The dogs had no edema, they lost markedly in weight, and the urine rarely contained a large amount of albumin or many casts. The lesions in the kidneys of the dogs suggest strongly that if the experiments could have been continued for a longer time dysfunc-

tion of the kidney more like that in the patient might have developed. It is, however, impossible to be certain whether the staphylococcus was the cause of the nephritis in the patient.

The experimental production of a systemic disease through the devitalization and infection of teeth in dogs with an organism having elective localizing power removes, it seems to us, all objections to the acceptance of the theory of focal infection and elective localization as an important factor in the etiology of disease.

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ARSPHENAMINE—SOME FACTORS WHICH INFLUENCE ITS COLLOIDAL PROPERTIES*

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THE pentavalent 3-nitro 4-hydroxy and 3-amino 4-hydroxy phenyl arsinic acids, occur in well-defined crystals, the former exhibiting a characteristic dimorphism. When reduced to the trivalent arseno stage the crystalline character of the pentavalent acids is suddenly replaced by a strongly gelatinous tendency. The arseno compound is amorphous, insoluble in water, incapable of recrystallization, and its soluble acid salts, of which the dihydrochloride arsphenamine is best known, are resinous substances incapable of crystallization.

This suggests that the reduction is accompanied by a polymerisation of the molecules, forming aggregates with colloidal properties. The change in physical characteristics is accompanied by a marked increase in therapeutic activity, which makes the arseno compound one of the most indispensable agents in the treatment of protozoal infections, and its satisfactory preparation a matter of great importance to medicine.

The generally accepted formula for arsphenamine is HCl.R.As=As.R.HCl, in which R represents the substituted phenyl residue. This corresponds to the analytical results, and to the general course of analogous reductions, but it does not seem to account for the sudden substitution of colloidal for crystalloidal properties on reducing the arsenic acid, or for the variations in these properties with different methods of preparation. It probably represents the structure of the arsphenamine molecule reduced to its simplest terms, the extreme reactivity producing a polymerisation or association with the formation of large complexes. In this sense the formula for arsphenamine may be written thus: $(HCl.R.As=As.R.HCl)_x$. This would explain the generally colloidal properties by the size of the molecular aggregate.

The stimulus to the chemical investigation of arsphenamine and its derivatives resulting from the granting of licenses for the manufacture in this country, has led to the introduction of several products under the generic name of arsphenamine, and also to the proposal of various preparative methods calculated to improve the product chemically, biologically or economically. The decided differences in the readiness with which various preparations dissolve in water has often been noted without explanations being offered. The originally imported Salvarsan was a greenish yellow amorphous powder which gave a clear solution with methyl alcohol. When just moistened with water it

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gelatinized, but when sprinkled on the surface of a large amount of water at ordinary temperature it dissolved readily and completely. The first brand of American made arsphenamine was practically insoluble in methyl alcohol, and when added to water of room temperature, formed gelatinous lumps which required heating or boiling to effect solution. In 1919, Kober[1] proposed a modified preparative method which yielded a product insoluble in methyl alcohol and which formed a jelly with water of room temperature. Recently Christiansen[2] has prepared arsphenamine which he states forms a jelly with cold water, and subsequently reported a change in the preparative method which yields a more readily soluble product. Some of the preparations which will be described in this paper, are apparently insoluble in methyl alcohol and when added to water of ordinary temperature form stiff jellies. Others dissolve readily and completely in methyl alcohol, and quickly form thin solutions with water of ordinary temperature.

The possibility of differences in the chemical identity of arsphenamine preparations has been considered, and although at times suggested by the striking differences in their characteristics, it seems very remote. Nitrophenolarsinic acid is in every case the starting point for the reduction to the arsphenamine base and this acid is a very distinct chemical individual. The various methods of reduction and conversion into the dihydrochloride which have been used, are of such nature that chemical rearrangements are probably excluded. The products may therefore safely be assumed to be "structurally" identical, and this claim is made for all commercial arsphenamines as well as for the products of some of the proposed variations in method.

It is a well-known fact that in the generally adopted syntheses of arsphenamines, chemically pure products can only be assured up to the point of the arsenic acids. Both the base and the dihydrochloride of the arseno compound are thrown out of solution in an amorphous condition and cannot be recrystallized. The impurities which occur, however, seem to be neither of a character nor in a sufficient quantity to produce the obvious differences which are found in the final products.

An alternative explanation which remains for these variations is on the physical grounds of molecular condition; they consist in differences in physical state, not chemical form. These in turn may be produced by differences in the method of preparation, and the work of which this paper is a report, consists in varying the process and noting the effect of different factors on the character of the end product.

A study of the literature shows that a large number of different procedures have been used for the preparation of arsphenamine. The method which has come into extended use for its production on a large scale consists in the reduction of the nitrophenolarsinic acid by means of sodium hydrosulphite, conversion of the resulting arseno base into its dihydrochloride in methyl alcoholic solution, and precipitation by means of ether or acetone. This is the method described originally by Ehrlich.[3] It has been generally adopted on account of the comparatively complete descriptions of the process which are available, and because of the smoothness of the one stage reduction, convenience of

manipulations, ready recovery of solvents and precipitants, and generally satisfactory nature of the products and yields. When suitably conducted it leads to an arsphenamine identical with that prepared by the original manufacturers, readily soluble in water of room temperature with a minimum of gelatinous tendency and soluble also in methyl alcohol.

Practically all other methods of preparation which have been used are either suggested or described or covered in patents by Ehrlich and his collaborators in this field.

Nitrophenolarsinic acid may be reduced to the arseno compound either progressively, with the formation of intermediate reduction products, or directly in one step, with simultaneous reduction of the nitro and arsenical groups.

The progressive reduction depends on the use of selective reducing agents. Reduction of the arsenical group without affecting the nitro group may be accomplished by phosphorus and hypophosphorous acid, which act specifically. This reduction is described in a patent of the year 1914.[4] Calculated quantities of stannous chloride also reduce the arsenical group leaving the nitro group intact, according to a patent issued in 1914.[5]

For the specific reduction of the nitro group, forming the amido-phenolarsinic acid, the following methods have been described:

1) By sodium amalgam in methyl alcoholic solution,[6]
2) By sodium hydrosulphite in cold solution, avoiding excess of reducing agent,[7]
3) By ferrous hydroxide in alkaline medium,[8]
4) Ammonium sulfide has been employed but has the disadvantage of producing a combination of sulphur in the arsinic residue, which must be subsequently removed.[9]

The simultaneous reduction of both nitro and arsenic groups in one step may be accomplished by sodium hydrosulphite as already described, or by hypophosphorous acid, using potassium iodide as a catalyst, as described in a patent of the year 1914.[10] It has also been claimed that zinc and concentrated acids in presence of sulphurous acid produce a simultaneous reduction of both groups, which stops at the arseno stage.[11] An electrolytic reduction was described by Barth in 1914.[12] Besides these methods, an interesting synthesis was described by Kahn in 1912,[13] which depends on a condensation of two reduction products of nitrophenolarsinic acid. When molecular solutions of aminooxyphenylarsine and aminooxyphenylarsenoxide are mixed, one molecule of water is eliminated and the arseno compound is formed directly.

Methods of isolating the dihydrochloride of the arseno compound are less numerous than those described for the reduction of the arsenic acid. There are in fact only two general methods, both described in the older literature. The first and generally adopted procedure consists in forming a solution of the dihydrochloride by adding the calculated amount of hydrochloric acid to a methyl alcohol suspension of the base, and precipitating the dihydrochloride from the solution by means of ether, acetone, or other anhydrous precipitants.[14] The second method is described in a patent of the year 1914;[15] and depends on

the insolubility of the dihydrochloride in an excess of aqueous hydrochloric acid. Here the aqueous solution of the reduction product is run into a large volume of aqueous hydrochloric acid, and the dihydrochloride may be obtained without preliminary isolation of the base, and without the use of organic solvents.

A number of these preparative methods have been applied recently to the laboratory production of arsphenamine by Kober,[16] Fargher and Pyman,[17] and Christiansen,[18] leading to products with variable physical characteristics.

With a view of comparing as comprehensively as possible, the effect of various methods of preparation in the properties of the final products, a series of experiments was laid out, covering 17 different arsphenamine preparations. The methods selected probably include all the essential variations in the known and practically applicable processes. Six of the above mentioned reduction procedures and the condensation process with arsine and arsenoxide were carried out. From these reduction products the dihydrochloride was prepared both by the methyl alcohol ether method, and by precipitation from aqueous hydrochloric acid.

In addition, some of the preparations made by the alcohol ether method were reprecipitated from aqueous hydrochloric acid, and some of the products obtained originally by the latter method were reconverted into the arseno base and then into arsphenamine by precipitation from ether.

It was observed, that arsphenamine prepared from the base by dissolving in a methyl alcohol solution of the calculated amount of hydrochloric acid, and precipitating by means of ether, was readily soluble in methyl alcohol, and also dissolved quickly in water, giving a thin limpid solution. The same arseno base, brought into aqueous solution as dihydrochloride, and precipitated from a large excess of aqueous hydrochloric acid, gave an arsphenamine which was insoluble in methyl alcohol and with cold water formed a jelly. One apparent exception to this rule was observed, in which precipitation from ether did not decrease the gelatinous nature of the arsphenamine, but this may have been due to certain other experimental factors. In general, the experiments show the behavior indicated above, with only variations in degree of difference between the two products. If an arsphenamine which dissolves readily in water of room temperature giving a clear thin solution, be reprecipitated by pouring its solution into an excess of aqueous hydrochloric acid, the resulting product no longer dissolves readily in water, but forms a viscous jelly. It also differs from the original by being insoluble in methyl alcohol. On the other hand, the difficultly soluble product, when reconverted into its base and then into the dihydrochloride in methyl alcohol, may be precipitated by ether, yielding a product readily soluble in water and methyl alcohol. This indicates that precipitation from aqueous solutions is a factor in increasing the gelatinous nature of arsphenamine, as compared with the material precipitated from anhydrous solvents. In other words the use of electrolytes for solution and precipitation increases the colloidal characteristics. This corresponds to one of the most striking properties of suspensoid sols, which are unstable in the presence of electrolytes. In certain concentrations the electrical charges of the suspensoid are neutralized

by the oppositely charged ions of the electrolyte, producing coagulation. There is a close parallel between this observation and the behavior of the arsphenamine solutions. Aqueous solutions of arsphenamine may be of the nature of suspensoid sols. When they are precipitated from solutions containing a large number of ions the neutralization of their surface charges produces coagulation or polymerisation which increases the gelatinous character of the dried product. These ionic effects are absent when non-ionized alcohol solutions and ether are used and the resulting products show a minimum of colloidal character. This explanation is offered tentatively to account for the wide variations in the physical characteristics of the different products described in this paper. Other possibilities must be considered on the basis of further study, which is being carried on. There is no doubt that other factors may also influence the character of arsphenamine, such as temperatures, purity of reagents, amount of mineral acid, mechanically retained moisture and impurities.

The above observations deal exclusively with variations in the characteristics of the solid arsphenamine and indicate their colloidal nature. The fact that arsphenamine solutions exhibit the characteristics of colloid sols has been noted recently. Bauer, in 1919, [19] using Bechhold's ultra filtration method, found that an alkalinized Salvarsan solution, after standing for some time, left a considerably smaller amount of residue on the filter than did the freshly prepared solution. This corresponds to the gradual decrease in the size and number of colloidal particles called peptization. The same author found that solutions made from commercial "Salvarsan Sodium," which is produced by precipitation from anhydrous media, left no residue when passed through the ultra filter.

Klemensiewicz, in 1920, [20] found that aqueous arsphenamine solutions exhibited viscosity phenomena which ranged them among the emulsoid colloids.

These physicochemical observations on the colloidal characteristics of arsphenamine solutions, as well as the variable characteristics of the dry arsphenamine described in this paper, appear to have a direct bearing on some of the biological and clinical observations which have been made in recent times. There have been noted wide variations in toxicity to experimental animals, sudden isolated cases of untoward clinical reactions from products which in the great majority of cases gave no trouble, as well as the toxic differences in different preparations. The large amount of purely chemical work which has been devoted to the question of the variability of arsphenamine has been singularly lacking in definite results. Although arsphenamine is not strictly speaking a chemically pure substance there is no known chemical impurity with the presence or proportion of which its toxic variability can be brought into connection. The only definitely recognized chemical substance to which extra toxicity can be ascribed is the so-called arsenoxide, resulting from the oxidation of arsphenamine. Ehrlich[21] first suggested the higher toxicity of this compound and the danger of its presence. Since then however, the consensus of opinion is that arsenoxide is neither so readily formed nor as highly toxic as was originally supposed. As a matter of fact, arsenoxide has never been demonstrated to be present in arsphenamine by actual isolation. This failure

of chemical investigation to bring to light a definite chemical impurity whose presence causes variations and extra toxic effects in arsphenamine, is of course not a proof of its absence. The symptoms shown by biological experiments on animals seem to indicate that the toxic effects are of different types.[22]

On the other hand, certain biological observations seem to correspond well with the results of physicochemical examinations of arsphenamine solutions. G. C. Lake found a decided decrease in the toxicity of alkalinized solutions on standing. A solution which was injected immediately after preparing, produced 14 fatalities in 15 rats injected in dosages of 120, 130, and 140 mg. per kilogram, 5 at each dosage. The same solution after standing for 30 minutes, produced only 2 fatalities at the same dosage and in the same number of animals. This almost inevitably suggests a connection with the results observed by Bauer, referred to above, in which he found the amount of colloidal particles in an alkalinized solution to decrease on standing. The recently communicated experiments of Reid Hunt,[23] who found that he could greatly reduce the toxicity of some arsphenamines by gently warming, or allowing the solutions to stand may also be explained by a so-called peptization of colloidal particles.

Whether or not these collodial properties of arsphenamine cause all or any of the variations and untoward effects of arsphenamine can only be definitely demonstrated by systematic coordination of physicochemical and biological research. But there is already sufficient evidence at hand to indicate a connection between the physical state of arsphenamine and its solutions, and its biological effects. The study of the differences in the state of dispersion of various arsphenamine solutions, their dependence on the type of product used, on the method of solution and alkalinization, on the age of the solutions, and the changes in the disperse state produced on introduction into the blood stream, seems at least as promising as further work along purely chemical lines, to explain the phenomena connected with the preparation and use of arsphenamine.

EXPERIMENTAL

Reduction I. Simultaneous reduction by sodium hydrosulphite.

This was carried on in the usual way, essentially as in the original description of the method.[3] 31.5 gm. of the nitro-phenolarsinic acid yielded 42 gm. of slightly moist arseno base. 28 gm. of this base was dissolved in 150 c.c. of methyl alcohol by the addition of 2.6 gm. of 100% hydrochloric acid in methyl-alcoholic solution. The resulting solution of the dihydrochloride was filtered, and divided into two equal parts, from which the arsphenamine was precipitated as follows:

Preparation No. 1.—One-half of the solution was precipitated by 900 c.c. of anhydrous ether, the precipitated arsphenamine filtered, washed with ether, and dried in vacuo. Yield—7 gm. slightly greenish yellow powder. Arsenic—31.57 per cent. The product gives a clear solution in anhydrous methyl alcohol, and dissolves quickly in water of room temperature, forming a thin limpid solution when made up to contain 6 per cent of the dry substance.

Preparation No. 2.—The second half of the solution was run with stirring into 500 c.c. of hydrochloric acid of approximately 15 per cent, which had been cooled to 0° C. After standing for about 10 minutes, the precipitate was filtered by suction, washed with a small amount of ethyl alcoholic hydrochloric acid to replace water, then with ether, and dried in vacuo over solid sodium hydrate. Yield—about 7 gm. Arsenic content—32.0 per cent, practically insoluble in methyl alcohol, gives a very viscous solution with 16 parts of water

at room temperature. On heating for a short time in the water-bath, this solution becomes thin, and remains so after cooling.

Preparation No. 3.—The remaining 14 gm. of base was dissolved in caustic soda and converted into the dihydrochloride essentially as described by Kober. Yield—about 6 gm. of dihydrochloride. Arsenic content—31.77 per cent, practically insoluble in methyl alcohol, solution in 16 parts of water at room temperature very viscous, becoming thin when heated and then remaining thin and limpid on cooling.

Reduction II. Simultaneous reduction by means of hypophosphorous acid and potassium iodide.

Preparation No. 4.—This reduction was carried out as suggested in D. R. P. 271894. Since Fargher and Pyman note that they could not confirm the reduction of the nitro group, following this patent, the method will be described in more detail. Forty gm. of nitrophenol arsinic acid dissolved in 140 c.c. of glacial acetic acid, 100 c.c. water, and 100 c.c. of 50 per cent hypophosphorous acid. This was heated to 75° C. in about 20 minutes, with stirring, during which time a yellow precipitate of the arsenonitro compound separates. At this point a concentrated solution of 24 gm. of potassium iodide was added. A violent reaction occurred on the first addition and the precipitate increased, the temperature rising quickly to 105° C., heating being discontinued. The mixture was kept at about 100° C. with stirring, and after a total time of about 1½ hours, the solution became clear. It was then cooled to 20° C. and poured with stirring into 500 c.c. of concentrated hydrochloric acid at about 10° C. The precipitate was filtered, washed with ethyl alcoholic hydrochloric acid, then ether, and dried in vacuo. Yield—20 gm., pink in color, which deepens to brownish red, even after packing in evacuated ampules. Arsenic content—33.7 per cent. Required hot water to obtain a solution, forming gelatinous insoluble lumps with cold water. Insoluble in methyl alcohol.

The following reductions are of the progressive type, in which the nitrophenol arsinic acid was first reduced by means of ferrous hydroxide in alkaline medium, following directions given by Jacobs and Heidelberger[9], and the amino phenol arsinic acid isolated.

Reduction III. Reduction of the amino phenol arsinic acid by hypophosphorus acid and potassium iodide.

Twenty-three gm. amino-phenol arsinic acid was mixed with 125 c.c. water and 125 c.c. of 50 per cent hypophosphorous acid. After adding 12 c.c. of 5 per cent potassium iodide solution, the mixture was heated for one hour to 60° C. in a carbon dioxide atmosphere. The clear solution gradually became very thick and viscous. It was cooled, diluted with 500 c.c. water, and divided into two equal parts.

Preparation No. 5.—One-half of the solution was made slightly alkaline with sodium carbonate, whereupon the arseno base was thrown out as a green voluminous slimy precipitate. This was filtered by suction and washed with water, then dissolved in 50 c.c. methyl alcohol and a sufficient quantity of hydrochloric acid in methyl alcohol solution. The addition of the hydrochloric acid produced at first a strong gelatinization, which thinned out again on the addition of more acid. The clear red solution was precipitated by ether in the usual way. Yield—6 gm. The product was obviously very impure due to decomposition of the base, and was rejected for purposes of comparison.

Preparation No. 6.—The other half of the reduction solution was precipitated by pouring into a mixture of 1,000 c.c. hydrochloric acid (1.19) and 400 c.c. water, cooled to 10° C. The finely shredded precipitate was filtered by suction, washed, and dried in vacuo over caustic. Yield—6.5 gm. Arsenic content—31.7 per cent, insoluble in methyl alcohol, very viscous solution in water, 1-16. The aqueous solution behaved in the usual way, becoming thin on heating, and remaining so after cooling.

Reduction IV.

Twenty-three and four-tenths gm. aminophenol arsinic acid was dissolved in a mixture of 150 c.c. water, and 50 c.c. hydrochloric acid. 2 gms. potassium iodide were added and the solution saturated with sulphur dioxide. After standing one hour, air was blown

through the solution until no odor of sulphur dioxide could be noticed. It was then neutralized with ammonia and filtered. Sodium hydrosulphite was then added to the filtrate, and the mixture warmed to 60° C. for about one hour, until a test showed the reduction to be complete. The fine yellow base was filtered, and washed thoroughly. The yield of moist base was 32 gm.

Preparation No. 7.—One-half of this was dissolved in methyl alcoholic hydrochloric acid and precipitated by ether. The dry hydrochloride weighed 8 gm. Arsenic content—31.7 per cent. Characteristics are those of the usual product from nitro phenol arsinic acid by hydrosulphite reduction. Soluble in water of room temperature without apparent viscosity.

Preparation No. 8.—The other half of this lot of base was dissolved in sodium hydroxide and precipitated by hydrochloric acid in the usual way. Yield about 7 gm. Arsenic content—31.5 per cent, insoluble in methyl alcohol, viscous solution with water.

Reduction V. Reduction of amino acid to arsenoxide and completion by means of hypophosphorous acid.

Twenty-three gm. amino phenol arsinic acid dissolved in 150 c.c. water, 50 c.c. hydrochloric acid added and then a solution of 2 gm. potassium iodide in 20 c.c. water. The clear solution was saturated with sulphur dioxide and after one hour, was freed from sulphur dioxide by blowing air through, and filtered. To this solution, 100 c.c. of 50 per cent hypophosphorous acid was added and the mixture allowed to stand 2 hours.

Preparation No. 9.—One-half of reduction mixture was diluted to 450 c.c. and made slightly alkaline with sodium carbonate. The base was precipitated in yellow flocks which rapidly darkened and finally became dark green. After filtering and washing, it was dissolved in methyl alcoholic hydrochloric acid and precipitated by ether. The dihydrochloride was normal in appearance. Yield—6 gm. Arsenic content—32.1 per cent, almost clear, soluble in 10 parts of methyl alcohol. It dissolved in 16 parts of water forming a solution which showed a slightly viscous behavior, but not nearly so marked as in preparation No. 10.

Preparation No. 10.—The other half of the above reduction mixture was run into 500 c.c.. 1-1 hydrochloric acid, the precipitate filtered off, washed and dried in the usual way. Yield—7.5 gm. Arsenic content—31.5 per cent, insoluble in methyl alcohol. Very viscous solution with water 1-16.

Reduction VI. Reduction of the amino phenol arsinic acid by sodium hydrosulphite.

Twenty-three gm. of amino acid were dissolved in 120 c.c. water and 60 c.c. caustic soda 2 N. The solution was added to a solution of 250 gm. of sodium hydrosulphite in 1200 c.c. water, and the mixture warmed to 60° C. until reduction was complete (1½ hour). The yellow base was filtered, washed and divided into 2 parts.

Preparation No. 11.—One half was dissolved in methyl alcoholic hydrochloric acid in the usual way and precipitated from ether. Yield—8 gm. Arsenic content—29.8 per cent. Soluble with slight turbidity in methyl alcohol, soluble in cold water giving a very slightly viscous solution.

Preparation No. 12.—The other half of this base was dissolved in caustic soda, acidified with hydrochloric acid, and precipitated from 1-1 hydrochloric acid at low temperature. The product stuck badly to the filter paper and could not be recovered in a clean state. It was insoluble in methyl alcohol and formed a very gelatinous solution with cold water.

Reduction VII.

The next product was made following the procedure described by Kahn[13]. Attempts to prepare amino hydroxy phenyl arsine by the complete reduction of nitro phenol arsinic acid did not give satisfactory results. It was found, however, that good yields of arsine could be obtained by the reduction of arsphenamine.

Reduction of arsphenamine to the primary arsine.

Thirty grams of arsphenamine were dissolved in 300 c.c. of water and 75 c.c. hydrochloric acid. The solution was stirred and 50 gm. of zinc dust added gradually, adding 100 c.c. more hydrochloric acid in portions to keep up the evolution of hydrogen, keeping the temperature below 30° C. all the time. When the action was over, 300 c.c. water were added,

and the solution warmed on the water-bath, adding more acid and zinc dust until the solution became practically colorless. This was then cooled, sodium acetate in excess added, and extracted with ether. The ether extract was dried with sodium carbonate and evaporated, leaving a residue of 24 gm. of white crystalline solid whose characteristics corresponded to those of the primary arsine.

Eighteen and five-tenths gm. of this arsine were dissolved in 200 c.c. of ethyl alcohol and 100 c.c. 2 N hydrochloric acid. The clear solution was added to a solution of arsenoxide prepared as follows: 23 gm. amino phenol arsinic acid were dissolved in 200 c.c. water and 100 c.c. hydrochloric acid 2 N 6 gm. potassium iodide were added, the solution saturated with sulphur dioxide and allowed to stand 2 hours. After mixing, the solution was allowed to stand for 2 hours, and then divided into 2 equal parts.

Preparation No. 13.—One half of this solution was neutralized with sodium carbonate, the base filtered off, dissolved in methyl alcoholic hydrochloric acid and precipitated from ether. Yield—10 gm. Arsenic content—31.7 per cent, readily soluble in methyl alcohol, and in cold water, giving a thin, nonviscous solution.

Preparation No. 14.—The other half of the above solution was poured with stirring into 1000 c.c. of 1-1 hydrochloric acid at 0° C., the precipitate filtered, washed and dried. Yield—12 gm. Arsenic content—31.96 per cent, completely insoluble in methyl alcohol, and forms a thick gelatinous solution with 16-20 parts of water.

Preparation No. 15.—Nine gm. of arsphenamine, prepared as in experiment No. 1, were dissolved in 90 c.c. water. The resulting thin limpid solution was precipitated by pouring into 900 c.c. 1-1 hydrochloric acid at 10° C. The product was filtered, washed and dried in the usual manner. Yield—7 gm. Arsenic content—32.2 per cent insoluble in methyl alcohol, difficultly soluble in water giving a thick viscous jelly.

Preparation No. 16.—Six gm. of arsphenamine, prepared as in experiment No. 14, were dissolved in 400 c.c. hot water. After cooling, the solution was made alkaline with sodium carbonate, the base filtered off, washed, dissolved in methyl alcoholic hydrochloric acid and precipitated from ether. Yield—3 gm. Arsenic content—31.77 per cent, clear solution with methyl alcohol, soluble in 16 parts of cold water without noticeable viscosity.

Preparation No. 17.—Six gm. of arsphenamine, prepared as in experiment No. 3, were precipitated as above from ether, yielding about 4 gm. of a product which gave a slightly turbid solution in methyl alcohol, and showed only a slight viscosity when dissolved in 16-20 parts of cold water. Arsenic content—31.75 per cent.

SUMMARY

A number of different arsphenamines have been prepared by varying the process of reduction and precipitation. The method of reduction produces certain differences in the characteristics of the arseno base, in regard to stability and extraneous impurities. On conversion into the dihydrochloride, precipitation by means of ionized solutions yields in every case products whose gelatinous characteristics, as evidenced by the viscosity of aqueous solutions and insolubility in methyl alcohol, are much more marked than when the dihydrochloride is prepared by the use of anhydrous non-electrolytes. This may be explained by the fact that arsphenamine is inherently a colloidal substance, and that electrolytes in certain concentrations produce coagulation of the disperse phase of its emulsoid sols. These macroscopic observations on the colloidal variability of arsphenamine, suggest the occurrence of less obvious, but similar differences in the disperse state of solutions prepared for intravenous injections, and consequently have a direct bearing on biological and clinical results obtained by their use.

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III. THE VALUE OF THE REFRACTO-VISCOSIMETRIC PROPERTIES OF THE BLOOD SERUM IN CASES OF TUBERCULOSIS*

By Max E. Bircher,† M.D., Rochester, Minn.

THE viscosity and refractivity of blood serum and their intimate relation have been studied in syphilis and cancer.[2, 3] I further found a deviation from the normal in occasional cases of actinomycosis, blastomycosis, sarcoma, and tuberculosis. In order to observe such deviation in patients with tuberculosis, I made a special study of 160 patients at the Glenn Lake Sanatorium. The condition of the patients was carefully analyzed and classified by the staff of the sanatorium. The technic used in making this study was essentially the same as that applied in the previous studies.[2, 3]

The cases were divided for study, according to the stage of the disease, into four groups: (1) Arrested cases in which there had been no signs of active tuberculosis for at least three months, and the patients were in good condition; (2) stationary cases in which neither progress nor improvement was taking place; some of the patients in this group were apparently in good condition; others were in an advanced stage of the disease; (3) cases in which the objective signs of tuberculosis were diminishing, and the patients "improving"; this group comprised patients who were still very sick, and others whose symptoms might soon be arrested; and (4) cases in which the signs of activity were of a progressive type. The four groups are divided in the chart by horizontal lines and subdivided by vertical lines.

Viscosity is expressed in relation to the viscosity of distilled water. In this series the large model of the viscosimeter of Hess was used, whereby a constant temperature of 20° C. was maintained.

Refractivity is expressed in the units of Pulfrich.[3]

Globulin percentage was obtained by interpolation with the aid of Naegeli's chart.[3] This percentage claims only relative value for reasons explained in previous papers.

The refractoviscosimetric quotient attributes to each serum a certain index which is obtained through division of the refractive index (multiplied by ten) by the viscosimetric index. It illustrates the fact that serum, respectively, its colloidal component with a certain refractive power may vary in viscosity. Two serums, for example, were obtained with a refractive index of 60.0 (Pulfrich units). One showed a viscosity of 2.00, the other of 1.80. The refractoviscosimetric quotient is 300 in the first sample and 333 in the second; the higher the quotient the lower the viscous power of a certain colloid substance.

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The normal range in each condition is represented by a shaded field in the chart. This range is based on a combination of my findings and those mentioned in the literature.[5, 6] It should be stated especially that the upper and lower limits are not absolutely sharp. The height of the black columns indicates the number of cases.

DISCUSSION

The viscosity is normal in arrested cases in Group 1. Although the other three groups comprise cases in which the viscosity is normal, there is a marked preponderance of cases in which the viscosity is high. In the pro-

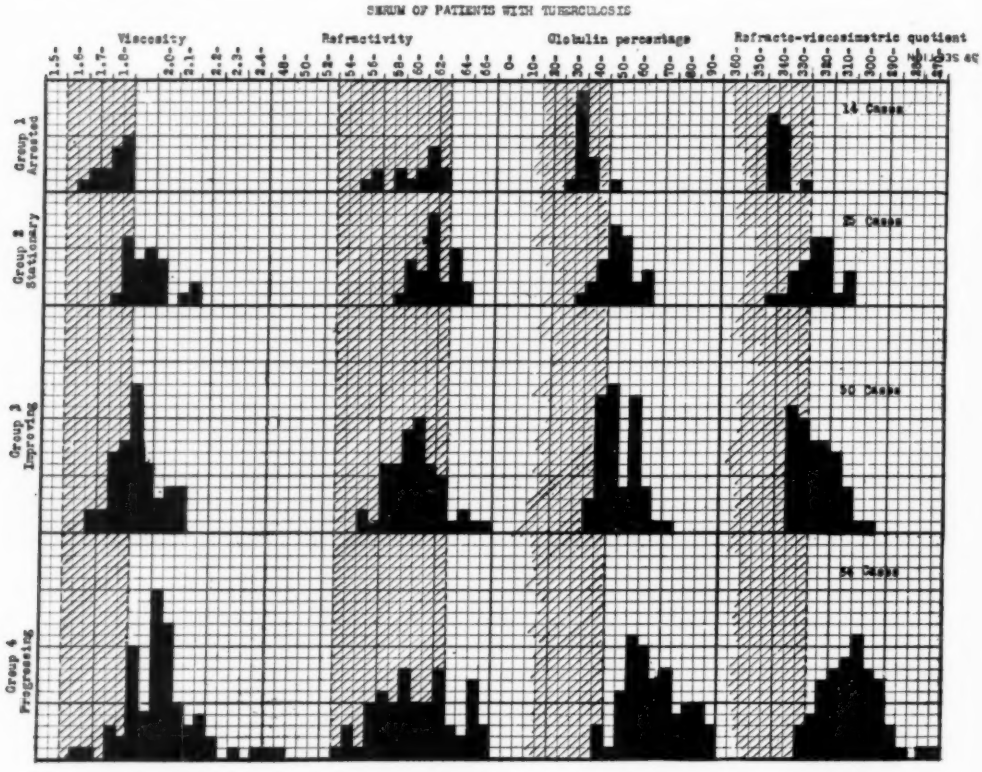

Fig. 1.

gressive stage of the disease (Group 4) the viscosity is greatly increased. Almost one-half of all the serums of this group have a viscosity between 2.00 and 2.10. A viscosity of 2.30, 2.40, and even of 2.60, as found in several instances, is to be considered as exceptionally high. Such degrees of viscosity were not observed in the great number of determinations made during the past year in other diseases. It is also worthy of note that in the few cases of Group 4 in which the viscosity was normal, the refractivity was extremely low. A viscosity of from 1.40 to 1.60 under usual conditions would correspond to such low refractive indexes. If, therefore, a viscosity of from 1.70 to 1.90 is found, this "normal" viscosity is very much elevated.

The refractivity of tuberculous serum is normal in most cases. The field

between 54 and 64 (Pulfrich units) corresponds, according to Reiss, to serums containing from 7 to 9 per cent of dissolved protein. Between these two percentages, Reiss declares, every normal serum may be ranged. It is, therefore, probable that the total amount of protein in the serum of tuberculous patients is not increased, or only slightly. This would indicate that the protein has a greater viscosimetric effect, or that it is not of a normal composition. The change that occurs in the protein is not explained by these tests. On the basis of the experiments of Naegeli and his collaborators it may be a change in the ratio of albumin and globulin. It is known that the viscosity responds very easily to a change in this ratio, while the refractivity is but little influenced by it.

The albumin-globulin ratio, as obtained with the aid of Naegeli's chart is reproduced in the third column, whereby only the globulin percentage is shown. As expected, the globulin is high, especially in cases of progressive tuberculosis.

The refractoviscosimetric quotient gives a very similar picture. It indicates that in tuberculosis the viscosity of the serum is abnormally high in relation to the refractivity. The refractoviscosimetric quotient classifies a number of cases as abnormal which in the first column showed normal viscosity. This is especially true in cases of Group 4. There are some exceptions. Three of the sixty-four patients with progressive tuberculosis showed normal refractoviscosimetric quotient. It was not possible to find a satisfactory explanation for this. One patient, however, had lost all resistance to the infection. On the other hand, the lowest values belong to patients in whom the disease is rapidly progressing and fatal. In Groups 2 and 3 half of the patients had normal rates. According to our classification these patients are in better condition than those with lowered rates. It did not seem advisable to subdivide the groups further on account of the limited number of cases.

Of the 160 patients examined the findings of 153 are discussed. The other seven patients did not have tuberculosis, but it should be noted that a decreased refractoviscosimetric quotient was found in those affected with blastomycosis, severe bronchiectasis with abscess, maxillary and other sinuses, recent catarrhal infection, and lead poisoning. In one case of peritonitis the refractoviscosimetric quotient was normal, in another it was definitely low. In tuberculosis the refractoviscosimetric quotient of the serum has a definite relation to the stage of the disease. The severity of the disease finds its expression in an accordingly low rate.

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THE ACTIVATION OF AN ENZYME POISONED BY HEAVY METAL SALTS*

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I. INTRODUCTION

IT HAS been shown, previously, that the coagulation of gelatin, through the action of various heavy metal salts, is reversible[1]; that by means of properly chosen salts of the alkali and alkaline-earth metals, the coagulated protein may be brought back to its original state. Since the toxic effects of heavy metals upon protoplasm is generally considered to be due to the coagulation of the proteins, and since the coagulated proteins may be restored to their normal physical state, it seemed reasonable to think that this end should be sought in a rational therapy devoted to combating the effects of heavy metal poisons. However, the explanation of the beneficial effects of the administration of neutral and alkaline salts in heavy metal poisoning can be attributed to this action of these salts, only insofar as the restoration of protoplasm to its normal physical state includes the resumption of its physiologic functions. The activity of the enzymes is so characteristic of normal protoplasmic behavior, that the idea suggested itself to me that like protein some typical enzymatic reaction mixture ought also to be capable of being "poisoned" by heavy metals and subsequently capable of "cure," through the addition of properly chosen lighter metals.

The following experiments, carried out upon saliva, show that this secretion behaves like the inert protein of our previous experiments, in that properly chosen neutral salts of the alkali and alkaline-earth metals restore it to its normal appearance and activity, after its coagulation and complete inactivation, through the action of heavy metal salts.

II. EXPERIMENTS

Fresh saliva was obtained by chewing paraffin, the same sample being employed in each series of related observations. A two per cent starch paste, made in the usual way from a commercial corn starch, was used as the substrate. The activity of the enzyme on the starch was tested qualitatively with Fehling's solution, after incubation of the substrate-enzyme mixture in a water bath at 40° C. Quantitative estimations were made with Benedict's quantitative solution, the amount of residual blue color being used as an index to the relative quantities of reducing sugars present.

1. *Inactivation of Saliva.*—When mercuric chloride is added to saliva up to a final concentration of $\frac{1}{200}$ M. a heavy flocculent precipitate is obtained,

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surmounted by a somewhat opalescent fluid. Lower concentrations of mercuric chloride produce less precipitate which also settles out more slowly. Higher concentrations of mercuric chloride produce a more rapid and complete settling out of the coagulum, so that the supernatant fluid becomes almost clear. In the lower concentrations of mercuric chloride the starch splitting ferments are inhibited. In any concentration of mercuric chloride above $\frac{1}{200}$ M. they are completely inactivated. Complete coagulation and inactivation requires from half an hour to an hour at room temperature, at the end of which time the heavy metal is firmly bound to the material composing the precipitate, as proved by the fact that the precipitate obtained

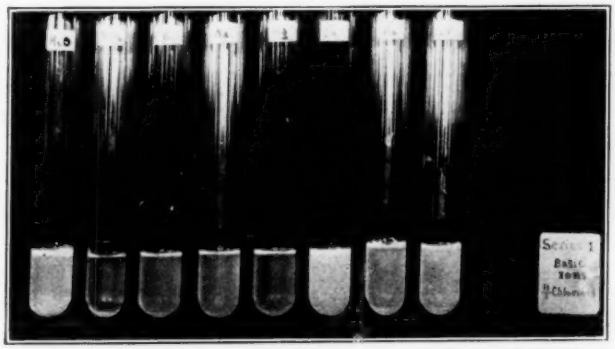

Fig. 1.

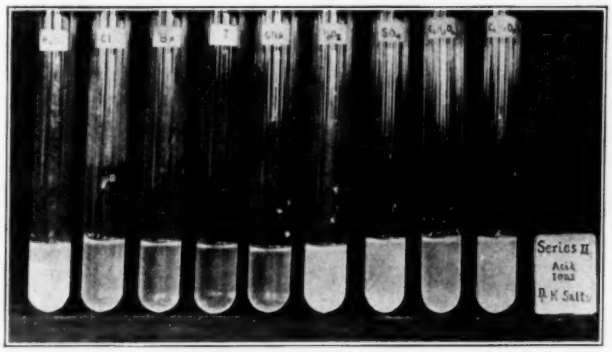

Fig. 2.

from 150 c.c. of saliva with mercuric chloride at a final concentration of $\frac{1}{200}$ M. in saliva, when filtered off and washed repeatedly with distilled water, failed to darken a bright copper wire placed in the dialysate obtained from the precipitate. Mercury could not be detected in the water surrounding the capsule though dialysis was continued for twenty days.

2. *Resolution of Mercurialized Saliva.*—(1) At various times (one hour to three months) after mercurial inactivation of saliva, small quantities of it were treated with various concentrations of neutral and alkaline salts of the alkali and alkaline-earth metals (after thorough shaking so as to obtain a homogeneous suspension). When sufficiently high concentrations of the chlo-

rides, bromides, iodides and sulphocyanates of ammonium, potassium, sodium, strontium, barium, calcium and magnesium were employed (omitting those not sufficiently soluble) most of the precipitate "dissolved" and the mixture again became a homogeneous opalescent liquid. The nitrates, sulphates, citrates, acetates and carbonates had little or no ability to produce these changes. The salts of strontium, barium and calcium caused a secondary precipitation of a white inorganic salt (sulphate?) along with the resolution of the original precipitate. Simultaneously with the resolution of the precipitate the starch splitting activity of the mixtures was restored. Those salts (nitrates, sulphates, citrates, acetates and carbonates) which did not resolve the precipitate, did not reactivate the enzyme. While resolution of the precipitate and reactivation of the ferment began quickly, restoration to normal appearance and behavior did not occur until several hours had passed.

(2) The speed and degree of the restoration of the ferment were affected by the concentration of the salt employed as the inactivator, and by the concentration of the salt employed as the reactivator. At a concentration of $\frac{1}{200}$ M. mecuric chloride in the mixture, the addition $\frac{1}{10}$ N. salt of the alkali or alkaline-earth metals produced little or no effect. Normal concentrations of such salts, when added to the inactivated saliva, produced the marked effects seen in Figs. 1 and 2. However, when the concentration of mercuric chloride was raised to $\frac{1}{50}$ N. or more, normal concentrations of the lighter salts had only a slight effect in resolving the precipitate, and no effect in activating the enzyme.

(3) The effects obtained by the use of the salts of the alkali and alkaline-earth metals were not due to a precipitation of the heavy metal in insoluble form, but to a displacement of the heavy metal from its combination with the material in the saliva, leaving the heavy metal in a soluble, diffusible state. This was shown by a heavy coating of mercury deposited upon a bright copper wire placed in the liquid surrounding the dialyzing capsule and contents

TABLE I

EFFECT OF BASIC RADICALS IN A SERIES OF CHLORIDES
STARCH SPLITTING ACTIVITY OF MERCURIALIZED SALIVA AFTER THE ADDITION OF A SERIES OF CHLORIDES

	TUBE CONTENTS	APPEARANCE AFTER 24 HOURS	COLOR OF ADDED BENEDICT'S SOLUTION
1	5 c.c. $HgCl_2$-saliva mixture + 5 c.c. H_2O	Turbid, opaque with precipitate unaffected	Original blue unchanged
2	5 c.c. " " " + 5 c.c. N NH_4Cl	Homogeneous—almost clear	Yellowish green; almost complete reduction
3	5 c.c. " " " + 5 c.c. N KCl	Homogeneous—less transparent than (2)	Pale blue
4	5 c.c. " " " + 5 c.c. N NaCl	Homogeneous—less transparent than (3)	Blue—deeper than (3)
5	5 c.c. " " " + 5 c.c. N $MgCl_2$	Practically same as (4)	Blue—very slightly deeper than (4)
6	5 c.c. " " " + 5 c.c. N $CaCl_2$	Secondary white precipitate	Yellowish blue—deeper than (2)
7	5 c.c. " " " + 5 c.c. N $BaCl_2$	Secondary white precipitate	Almost the same but slightly paler than (6)
8	5 c.c. " " " + 5 c.c. N $SrCl_2$	Secondary white precipitate	Paler than (7)—deeper blue than (2)

described above, within a few minutes after the introduction of 10 c.c. of molar potassum bromide into the dialysis capsule.

(4) The salts which bring about restoration of the enzyme are not all equally effective. The following experiment shows this and serves to illustrate the methods of our quantitative experiments. Mercuric chloride was added to fresh saliva up to a final concentration of $\frac{1}{200}$ M. in the mixture. Twenty-four hours later the mercurialized saliva was shaken thoroughly and introduced into test tubes in 5 c.c. amounts. The subsequent treatment of these tubes is shown in Tables I and II and Figs. 1 and 2. Twenty-four hours after the additions indicated, 0.5 c.c. of the contents of each tube was removed and mixed with 5 c.c. of two per cent starch paste, in small tubes of uniform thickness and diameter. After being carefully shaken in a rack and introduced simultaneously into a water bath at 40° C. for half an hour, they were cooled in cold tap water for a few minutes. One c.c. of the topmost (clear) fluid of each tube was quickly transferred to small tubes of the same thickness and diameter, and to each tube 5 c.c. of Benedict's quantitative sugar reagent were added from a burette. The tubes were shaken in a rack for a few seconds to insure admixture, immersed simultaneously in a boiling water bath, and after 20 minutes were subjected to a color comparison by means of the unaided eye, the residual blue color of the unchanged cupric salt being used as the reciprocal index to the relative quantities of the reduc-

TABLE II

EFFECT OF ACID RADICALS IN SERIES OF POTASSIUM SALTS
STARCH SPLITTING ACTIVITY OF MERCURIALIZED SALIVA AFTER THE ADDITION OF A SERIES OF POTASSIUM SALTS

	TUBE CONTENTS	APPEARANCE AFTER 24 HOURS	COLOR OF ADDED BENEDICT'S SOLUTION
1	5 c.c. $HgCl_2$-saliva mixture + 5 c.c. H_2O	Turbid, opaque with precipitate unaffected	Original blue unchanged
2	5 c.c. " " " + 5 c.c. N KCl	Homogeneous, slightly opaque	Pale blue
3	5 c.c. " " " + 5 c.c. N KBr	Homogeneous, almost transparent	Yellow, practically complete reduction
4	5 c.c. " " " + 5 c.c. N KI	Homogeneous, practically same as (3)	Faint greenish yellow
5	5 c.c. " " " + 5 c.c. N KCNS	Homogeneous, slightly opaque	Blue, slightly deeper than (2)
6	5 c.c. " " " + 5 c.c. N KNO_3	Same as (1)	Original blue unchanged
7	5 c.c. " " " + 5 c.c. N K_2SO_4	Same as (1)	Original blue unchanged
8	5 c.c. " " " + 5 c.c. N $KC_2H_3O_2$	Slightly clearer than (1)	Original blue unchanged
9	5 c.c. " " " + 5 c.c. N $KC_6H_5O_7$	Same as (1)	Original blue unchanged

ing sugars formed. (Only differences obvious to the unaided eye were considered significant.) The uniform results obtained from several repetitions of the experiment are recorded in the last column of Tables I and II. A tabulation of these results indicates the relative values of the various radicals, in the order of their potency in restoring the poisoned enzyme to be as follows, that most powerful being mentioned first:

Basic Radicals	*Acid Radicals*
Ammonium	Bromide
Strontium	Iodide
Calcium	Chloride
Potassium	Sulphocyanate
Sodium	
Magnesium	

(5) The enzyme coagulated and completely inactivated through the action of mercuric chloride may be restored to 80 per cent of its normal starch splitting activity. Equal quantities of fresh saliva and of mercurialized saliva reactivated after forty-eight hours with ammonium chloride were allowed to react upon starch paste as above, and color comparisons were made quantitatively by means of a Bock-Benedict colorimeter. Repeated experiments showed a uniform restoration of the previously inactivated enzyme to 80-85 per cent of the efficiency of the fresh saliva.

3. The foregoing experiments were repeated in part using silver nitrate as the inactivating agent. This salt coagulated and completely inactivated saliva at a final concentration of $\frac{1}{50}$ N. in the saliva. In general, the results of the experiments were the same as those obtained in the case of mercuric chloride except that higher concentrations of the light metal salts were necessary to produce the effects.

The common lead salts proved to be incapable of completely inactivating the salivary ferment, so that experimentation with them was abandoned.*

III. DISCUSSION OF RESULTS

In explanation of the restoration of coagulated proteins to their normal physical state, through the action of light metal salts, we expressed the view that the process consists in the conversion of insoluble, unhydrated heavy metal protein compounds into more soluble hydrated light metal protein compounds;[1] that these compounds of metals and proteins may be considered analogous to soaps formed by the combination of heavy and light metal oxides and hydroxides with fatty acids; in that the heavy metal soaps are uniformly slightly soluble and slightly hydrated, while the light metal soaps are soluble and highly hydrated.[2] The extension of this conception to the explanation of our observations on saliva, necessitates the following conclusions:

(1) That the enzyme is a protein, or that it is intimately associated with a protein, which is soluble and active only when combined with certain salts or metals.

(2) That it is capable of combining with other inorganic salts, so as to form a series of metal-protein-acid compounds, analogous in general, in solubility and hydration capacity (though not necessarily exactly so) to the soaps formed by the neutralization of fatty acids with metallic hydroxides.

(3) That such heavy metal salts as inactivate the enzyme, do so through the formation of insoluble, dry, heavy metal-protein-acid compounds, thus removing the active catalyst from the solution.

(4) That such lighter metal salts as reactivate the inert enzyme do so

*Incomplete experiments with pepsin indicate that this enzyme may be reactivated after complete inactivation through the action of mercuric chloride.

by replacing the heavy metal of the compound with the light metal of the introduced salt, forming a new compound—a soluble and hydrated and hence active light metal-protein-acid compound.

(5) That the effect of the acid radicals is due to their combination with the protein to form various compounds differing from each other in solubility and hydration capacity.

The facts obtained in our experiments and in those of others are in agreement with these conclusions. That the enzyme is not active except in the presence of various salts is shown by the observation of numerous investigators,[3] that dialysis completely inactivates the salivary enzyme, (a precipitation occurs in the dialysis tube) but the addition of any one of several neutral salts almost immediately restores the ferment to its activity, (this being accompanied by resolution of the precipitate obtained in dialysis). This may be interpreted as meaning that the active ferment is a metal-protein-acid compound, which upon hydrolysis yields an insoluble protein as a precipitate in the dialysis tube.

That stabile compounds of the enzyme with heavy metals are formed when the saliva is treated with heavy metal salts, is strongly suggested by the fact that the precipitate so obtained does not release the metal after long dialysis.

That a new compound is formed, and the heavy metal freed from combination when the proper salt is added to the heavy metal enzyme precipitate, is indicated by the observation that such treatment causes an almost immediate restoration of the starch splitting activity of the enzyme, and a simultaneous diffusion of the hitherto undiffusible heavy metal through a dialysis tube.

It should be pointed out that the impurity of the enzyme (or enzymes) employed in the experiments must be taken into account in a comparison of quantitative results. The presence of salts other than those added experimentally, especially such as cause precipitation in the mixture, cannot be without effect. This factor alone may explain the apparent abnormal position of certain of the alkaline-earth metals in the table of effective salts. It is also probable that proteins present in saliva other than the active enzyme (presumably a protein) have definite effects on the behavior of the salts added and on the enzyme itself. The manifest difficulty of obtaining a pure ferment has up to the present time prevented our taking adequate account of these factors.

IV. PRACTICAL APPLICATIONS

Whether or not our explanation of our observations is correct, the facts without explanation are important not only for a better understanding of the nature and behavior of enzymes, but for the better therapy of heavy metal poisoning in living tissue. Our original question as to whether living material coagulated by heavy metals could be restored to function as well as to normal physical state through the action of salts of lighter metals may be considered answered. This brings us to restate a conclusion based upon earlier experimentation, that in the treatment of heavy metal poisoning, our efforts should be devoted chiefly to the restoration of the coagulated tissue

to its normal state and to maintaining it in this normal state till the injurious agent shall have been removed.

Our experiments indicate the probability that not only can tissue coagulated by heavy metals be restored to normal state and behavior but that the heavy metal can be hastened out of the body by maintaining it in soluble and diffusible form.

Neither is there a lack of clinical[4] and experimental[5] evidence that these results can be accomplished.

A restatement of the method to be employed in the treatment of individuals poisoned by heavy metals may not be amiss. In order to combat the primary coagulative effects of heavy metals upon the tissues it is necessary to obtain, at the earliest possible time, a high concentration of suitable salts in the body and to maintain this high concentration over a long period of time. The salts which can best be employed for this purpose are the salts of potassium and sodium. Because of the buffer action of the tissues of the body and because of the ready oxidation and elimination of such organic acids as citric, acetic and carbonic the objection to the use of salts of these acids, as would be concluded at first thought from the examination of the experimental data, will not obtain. On the contrary these are the ones which should be employed because they are easily borne at high concentrations in the body. In addition these salts will serve best to combat the secondary "acidosis" which is the constant accompaniment of heavy metal poisoning. The alkaline salts of sodium and potassium, then, should be administered in large doses and should be maintained in the body up to the limit of tolerance, till such a time as the heavy metal has been excreted from the body.

V. SUMMARY

1. The enzyme in saliva inactivated (coagulated) through the action of the heavy metal salts $HgCl_2$ and $AgNO_3$ may be reactivated by means of sufficiently high concentrations of certain neutral salts of the alkali and alkaline-earth metals.

2. The degree and rate of reactivation of the coagulated enzyme is affected by the concentration of the coagulant and the reactivating salts and by the basic and acid radical of the salt employed as the reactivator.

3. Facts are set forth, indicating the probable protein nature of the enzyme, together with the probability of the formation of various compounds of salts with this protein.

4. A restatement is made of the principles underlying a rational therapy of heavy metal poisoning.

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THE EFFECT OF BILE SALTS IN THE URINE ON ROUTINE TESTS FOR ALBUMIN*

By Symmes F. Oliver,† M.D., Cincinnati, Ohio

IN THE course of some investigations on bile, I became interested in the urinary findings of patients suffering from various diseases of the liver and the biliary passages.

My attention was early directed to the frequency with which albumin is reported in the urine of such patients, particularly those suffering from jaundice of hepatic origin. Often, albumin is reported without casts or other evidences of renal disease. In cases of common duct obstruction, urinalysis almost invariably showed albumin while text books discussing the question, speak of albuminuria as a frequent complication.

Routine urinalysis performed on a large number of cases of obstructive jaundice revealed the following: Practically all gave positive tests for albumin with the acetic acid and heat tests, Heller's cold nitric acid test and Robert's reagent. In a few cases a double ring, one just above the other, was obtained with HNO_3 and with Robert's reagent. These rings formed immediately and became stronger on standing. The upper ring on standing tended to diffuse upward so that after about one hour, the whole upper layer of urine became clouded. The lower ring, exactly at the point of contact between the reagent and urine remained constant or became slightly accentuated. The occurrence of this double ring suggested that two different substances were present in the urine, which reacted with the reagents used. The lower ring exactly at the point of contact of the reagent and urine was found to be albumin. The upper ring was not due to urates since this ring only forms on standing for some little time, and tends to appear higher up in the urine than the ring under discussion. Likewise, urates do not interfere with the acetic acid test whereas the substance in question caused a decided clouding without the application of heat. Further, the urate ring clears up when heat is applied while the ring here described is not affected. Mucin and various nucleo-albumins may, under certain conditions, give reactions that lead to confusion. These substances may be ruled out, however, by testing for protein compounds and, if present, removing them from solution. Mucin causes a slight clouding with acetic acid and occasionally is present in sufficient concentration to produce a ring with nitric acid—or Roberts reagent. This ring, however, is a fine delicate crystalline ring which easily might escape detection. Mucin rings may be obtained for comparison by using nasal secretion where mucin exists in almost pure form. If mucin is suspected, dilution of the urine together with filtration would eliminate it as a factor. The ring described in this paper is a broad milky one just above the layer of contact between the

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reagent and urine and tends to diffuse rather rapidly upward, ultimately clouding the entire urinary layer. In all cases where such reactions are obtained it would be advisable not to too readily assume that mucin and nucleo-albumins are the responsible agents.

Since the urines examined were all obtained from cholemic patients, it seemed logical to suppose that certain constituents of the bile might be the factors responsible for these changes. It is known that bile salts are precipitated from solution when treated with mineral acids and dilute acetic acid and that they can be redissolved on the addition of alkali. The usual reagents used in the common tests for albumin, such as nitric acid, hydrochloric acid and weak acetic acid, all bring about this change. Solutions of bile salts were made and tested with the above reagents. In all cases a ring such as described above, formed just above the layer of contact of the reagent and solution used. It was found that bile salts in dilutions as high as $\frac{1}{64}$ of 1 per cent gave positive ring, and acetic acid tests. Small quantities of Sodium glycocholate and taurocholate were then added to normal urine and tests identical to the above were obtained.

Urines that were known to contain albumin were then treated with traces of bile salts and tested. Double rings such as previously described were obtained in all specimens examined.

The urines examined were then in all cases tested for bile salts. Four tests were used for each specimen, namely: Hay's sulphur test, the peptone test, the precipitation of egg albumen and the starch reaction. The latter two tests consisted in the use of 2 per cent egg albumen and starch solutions, weakly acidified with acetic acid and preserved with small amounts of salicylic acid. These reagents are rendered milky or definite precipitation occurs in the presence of bile salts. In the latter three tests, the urine was diluted so that the specific gravity was reduced to 1008, filtered if necessary and rendered faintly acid with acetic acid. Four c.c. of the reagents were used and the urine added drop by drop until a milkiness of the solution resulted. In all cases where these tests were made, a decided increase in bile salt content was found in the urine. In this way a check on all urines was obtained so that other ingredients could be excluded. In all cases of obstructive jaundice and the majority of cases of cirrhosis of the liver, a bile salt increase was found in the urine. It is noteworthy that in certain cases of cirrhosis of the liver, a decided increase in bile salt was found without any increase in bile pigment. Conversely, certain of these cases showed an increase in bile pigment with little or no augmentation of bile salts.

In all cases where an increase in bile salts was noted in the urine, it was observed that the urine was cloudy and turbid, if the reaction of the mixture was acid. This turbidity was increased if acid were added to the mixture. Neutral or alkaline urines were found to be almost always clear.

Roughly, this turbidity of acid urines was proportional to the bile salt content present. After cholecystostomy or cholecystectomy the urines usually cleared up within four to six days after operation. The urine becomes lighter colored, the bile salts decrease in amount and the turbidity no

longer occurs in acid urines. A study was made in this connection on the urinary findings before and after operations on the gall bladder, and the findings will be reported later. It may be briefly stated that the bile salt content of the urine was found to be dependent on the secretion of bile by the liver and the concentration of bile salts in the bile. In cases of hepatic insufficiency it was found that an increase in bile salts occurred in the urine, whereas in cases where the functional activity of the liver soon regained its normal balance, the bile salts likewise tended to disappear from the urine and appear in normal amounts in the bile. These findings, however, will be reported in detail in a subsequent communication.

In conclusion, the clinical importance of these findings is quite obvious. It is of decided importance to the clinician to know whether he is dealing with an ailment which is hepatic in origin or whether the pathology is indicative of renal disease. Two cases, illustrative of this point, may be mentioned. One case, seen by Doctor Robert Kehoe, of the Department of Physiology, was a young woman who was six months pregnant. Her urine had been examined and albumin was reported positive. She had a slight elevation of temperature, together with digestive disturbances and evidences of toxemia. It was feared that she was developing eclampsia and the question as to the advisability of emptying the uterus came up. Doctor Kehoe noticed that she had a slight jaundice, and on examining the urine, found that no albumin was present but that there was a decided increase in bile salts. As a result of these findings, no operative interference was resorted to and the woman was able to save her baby.

The second case was a patient in our own practice: A woman had been operated on for gall stones, but, after the operation, her convalescence was extremely slow and tedious. She complained of excessive fatigue, prostration and absolute loss of appetite. The sight of food nauseated her and she was unable to retain her nourishment. Urinalysis revealed a urine of high specific gravity, with moderate amounts of albumin and a few casts. A decided increase in bile salts was also noted. At this time, however, I was unaware of the effects of bile salts on the albumin tests and the patient was consequently treated for nephritis. She received five intravenous injections of Fischer's solution with no improvement other than an increased excretion of urine. Clinically she was absolutely not benefited by her treatment and, after a lingering illness in which she became progressively weaker and more emaciated, she ultimately died. The use of alkaline therapy in cases of cholemia I believe is certainly not indicated and this case was probably actually harmed by the treatment she received.

Other cases might be mentioned, but these two suffice to show the practical importance of bearing these points in mind.

In conclusion, I wish to express my deep appreciation to Doctor Martin H. Fischer, of the Department of Physiology, for the opportunity to carry on this work. I also wish to thank Doctor F. B. Samson, of this city, for his helpful counsel and kindly advice, and Doctor A. P. Matthews and Doctor Shiro Tashiro of the Department of Physiological Chemistry, for their valued aid.

THE NATURE AND TREATMENT OF MUSCULAR ATROPHY*

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STATEMENT OF THE PROBLEM

THE invariable wasting of muscles which follows section of the motor nerve, or destruction of the anterior horn cells of the lower motor neurones supplying the muscle, has led to the theory that the nerve cell exerts a *trophic* influence on the muscle. Neither physiological experiments nor anatomical evidence has succeeded in demonstrating the nature of this trophic influence. By some writers, the influence is supposed to reside in special trophic fibers; by others, a peculiar kind of nerve impulse is assumed in order to explain the facts. Numerous observations have been made in which a trophic influence of the nervous system upon other tissues and organs than muscle, seemed to be demonstrated. The trophic ulcers of the cornea following injuries to the fifth nerve, and lesions of the bone such as Charcot's joint, are cited in this connection. Gradually however, evidence has accumulated to show that most of these phenomena can be explained in a more satisfactory manner upon other grounds. Again, it has been thought by some on the basis of Boecke's work[2] on the motor end plate, that there is a continuity of substance from the nerve fiber through the end plate to the muscle. Since the protoplasm is continuous from nerve to muscle the atrophy of the nerve is necessarily followed by the wasting of the muscle. Against this view, it may be urged that muscle and nerve cells react differently to vital stains and to drugs, so much so in fact, that the two tissues differ fundamentally from a physiological point of view. Furthermore, if more proof is needed, it is a well established fact that the denervated muscle retains its irritability to a faradic current long after such irritability is lost in the nerve.

There remains to be considered the time honored theory that the muscular atrophy following nerve injuries is due to disuse. In support of this view there is the undoubted fact that a muscle which is not used decreases in size and strength as is shown in man by the shrinkage of limbs paralyzed by an upper motor neurone lesion. The atrophy however, of a denervated muscle is much more rapid. Indeed, as the curve (Fig. 1) shown in this paper proves, agreeing as it does so closely with that of Langley, there must be some general law of physiology at work. A suggestion as to the nature of this law will be made later in this paper.

A practical consequence of great importance, of the disuse theory of atrophy is the treatment of atrophying muscle by electrical stimulation. Since 1841 following the lead of John Reid[13] it has been customary to treat mus-

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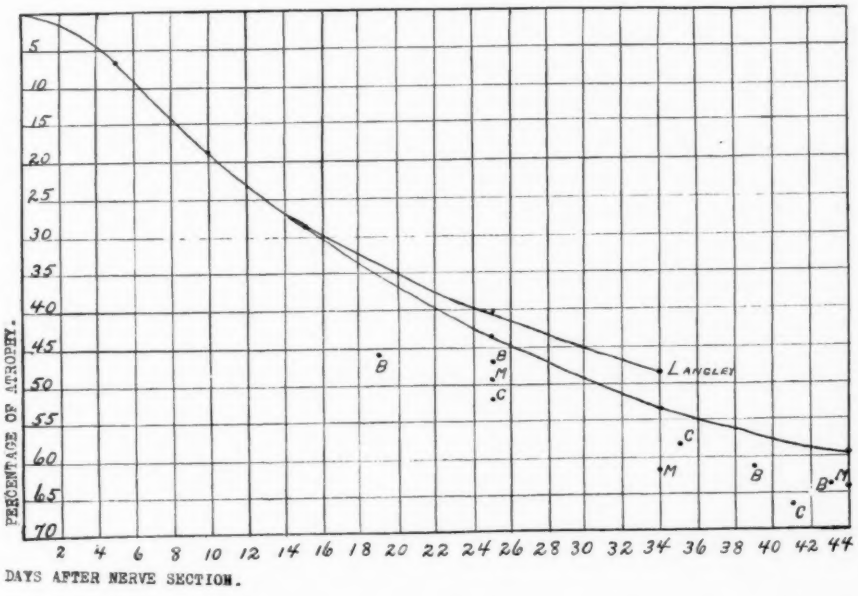
cular atrophy by causing the implicated muscles to contract by electricity, on the assumption that if this treatment did not prevent atrophy entirely, it would tend to lessen it. This traditional clinical opinion has again lately been voiced by Roberts.[14] Langley has reported a slight increase in size after long continued stimulation. Hartman and Blatz[17] in a recent paper were unable to demonstrate any beneficial effect from either galvanic stimulation or massage. At any rate, the influence of the electric current is probably not due to the production of contractions in the treated muscle as Cumberbatch[3] reports favorable results from the use of alternating sinusoidal currents which do not cause contractions.

A change of attitude towards the nature of muscular atrophy has recently come about through the work of J. N. Langley and his pupils. Langley and Kato[7] in the course of some experiments on the physiological action of

FIG. I



physostigmine had occasion to cut the sciatic nerve of rabbits. They noticed on the fifth day after nerve section that the gastrocnemius muscle was in a state of rapid, fibrillary contraction. These contractions are very small and can be seen best when the light falls at the proper angle on the wet, glistening surface of the muscle. This state of incessant activity persists throughout the whole period of muscular atrophy. This fact although recently confirmed by Langley was first described by Schiff[15] in 1851. In the course of experiments on the paralysis of the tongue in dogs following section of the hypoglossus on one side, Schiff observed the same fibrillary contractions in the atrophying muscles of the tongue. Schiff's language is so accurate and vivid that there can be no doubt that he was observing the identical fact described by Langley. Stevens[16] has confirmed and extended Schiff's experiment on the hypoglossus of dogs. The additional fact was brought out

by Stevens that the fibrillary contractions begin within three days after nerve section and persist throughout the whole period of atrophy, only to disappear with the regeneration of the nerve. There is therefore, an exact parallelism between the onset and duration of the atrophy and the appearance and continuation of the contractions. Beyond describing the fact, Schiff made no inference as to its significance. Langley however, impressed by the exact concomitance between the incessant fibrillar activity and the atrophy of the muscle has suggested that the former event is the cause of the latter. The wasting of a denervated muscle is really a fatigue effect caused by the ceaseless fibrillar activity. In support of this view of the facts Langley[4] and Barcroft and Kato[1] have shown that the specific gravity of atrophied muscle and fatigued muscle are less than that of normal muscle. Furthermore, as demonstrated by Langley and Itakagi[6] there is an increased use of oxygen in atrophied muscle in comparison with normal muscle, which they interpret to support the theory of fatigue by indicating an increased rate of breakdown.

OUR OWN EXPERIMENTS

The purpose of the experiments described in this paper is to test the theory that muscular atrophy is caused by incessant fibrillary activity. The particular method of attack here employed was suggested by certain experiments of Jacques Loeb[10] on the effect of certain ions on the rhythmical contractions of muscles. In the course of an investigation on the physiological activity of sodium and calicum ions, Loeb observed that skeletal muscles could be caused to contract rhythmically in a solution containing Na-ions. He used both NaCl and NaBr. The addition of a small quantity of Ca-ions or K-ions or both, inhibited these contractions. He had proved[9] earlier that Ca-ions decreased the excitability of skeletal muscles. Later[11] he established the fact that rhythmical contractions can be excited in smooth muscle by the addition of sodium ions and inhibited by calcium ions. He also showed[12] that a smaller amount of K- or Ca-ions are required to inhibit contractions in smooth muscle which contains no ganglia than in muscle which contains ganglia. The object of our experiments was to determine whether we could inhibit the fibrillar activity of denervated, skeletal muscle by the injection of certain salts of the Ca-group, and therefore prevent muscular atrophy. Langley has shown that the intravenous injection of calcium salts checked the fibrillar contractions. His experiment was not extended over a sufficient length of time to demonstrate any effect upon muscular atrophy.

The animals used in our experiments were guinea pigs. In each case, the right sciatic nerve was severed in the gluteal region and 6 mm. of nerve excised. Since accidental differences in the size of right and left groups of muscles are distributed according to chance, the possibility of a systematic error on this account is avoided by selecting the same leg for operation in all experiments. The possible influence of sex was left out of account. The wound healed always by first intention, the operation being usually bloodless. The animals were operated in groups of eight. Three pairs were then injected hypodermically with calcium, barium or magnesium salts, beginning the day

after the operation. It was found by experiment that the strongest concentration of salts which could be used without causing sloughing of the skin were $CaCl_2$m/4, $BaCl_2$m/10 and $MgSo_4$m/1. One cubic centimeter of the sterile aqueous solution of these salts was injected daily in the appropriate animal. Six guinea pigs were injected for 25 consecutive days; six for thirty-five days; and six for forty-four days, with some exceptions to be noted later. In each group of eight operated pigs there were two which served as controls by receiving no injection whatever. At the end of the time chosen for the test, the animals were killed and the gastrocnemius muscle on each side weighed. Each muscle was dissected out singly by the same person, in order to secure uniformity of dissection, and weighed as rapidly as possible on a sensitive balance, to avoid loss of weight from evaporation. The results of the experiments are shown in table I, in which the number of each animal in the series is given, together with the duration of the treatment, the salt used, the weight in grams of right and left gastrocnemius muscles, the difference in weights and the percentage of atrophy. The percentage of atrophy was calculated by dividing the weight of the left muscle into the corresponding difference of weight between right and left muscles.

The results of the experiments are also shown in graphic form in Fig. 1. The first part of the curve of atrophy is copied from Langley and Kato,[8] the latter part is drawn from our control pigs. The curve agrees with the con-

TABLE I

NO. PIG	DAY KILLED	INJECTED WITH	WT. OF RIGHT MUSCLE	WT. OF LEFT MUSCLE	DIFF.	% OF ATROPHY
1	44	Control	0.930	2.427	1.497	61.7
2	44	Control	0.722	1.701	0.979	57.5
3	43	$Mg.SO_4$m/1	0.872	2.441	1.569	64.2
4	45	$Mg.SO_4$m/1	0.652	1.852	1.200	64.6
5	38	Ca Cl_2m/4	0.692	2.102	1.410	67.1
6	44	Ca Cl_2m/4	0.752	2.302	1.550	67.3
7	43	Ba Cl_2m/10	0.756	2.331	1.575	67.6
8	43	Ba Cl_2m/10	0.725	1.831	1.106	60.3
9	34	Control	1.075	2.159	1.084	50.2
10	33	Control	0.515	1.657	0.938	56.6
11	33	Mg SO_4m/1	0.595	1.522	0.927	60.9
12	35	Mg SO_4m/1	0.756	2.013	1.257	62.4
13	35	Ca Cl_2m/4	0.955	2.377	1.322	55.6
14	35	Ca Cl_2m/4	0.630	1.575	0.945	60.0
15	39	Ba Cl_2m/10	1.931	2.342	1.411	60.2
16	19	Ba Cl_2m/10	1.080	1.992	0.912	45.7
17	25	Control	1.554	2.821	1.267	44.8
18	25	Control	1.025	1.772	0.747	42.1
19	25	Mg SO_4m/1	0.202	2.291	1.089	47.7
20	25	Mg SO_4m/1	0.659	1.331	0.672	50.5
21	25	Ca Cl_2m/4	0.927	2.077	1.150	55.4
22	25	Ca Cl_2m/4	1.977	1.876	0.899	47.9
23	25	Ba Cl_2m/10	1.246	2.340	1.094	46.8
24	25	Ba Cl_2m/10	1.066	2.005	0.939	46.8

clusions of Langley and Hashimoto[5] that the curve of atrophy should fall more sharply in the third week and is generally lower than Langley and Kato's curve. The observations are too few to plot definite curves for each

salt. In each case the points indicated are the averages of two pigs similarly treated, except in the case of Pigs 15 and 16, which are plotted separately because Pig 16 died suddenly on the nineteenth day, immediately following injection, and the autopsy failed to reveal any sufficient, anatomic cause of death. It is possible that it was injected directly into a vein. Also in the case of Pig 13, the injections were stopped on the fifth day because of an extensive slough at the point of injection. In spite of these slight irregularities, a glance at the figure shows that the degree of atrophy is invariably greater in the injected pigs than in the uninjected animals.

While the results of these experiments are negative with respect to the question which they sought to answer; viz., can the fibrillary contractions of denervated muscle be stopped, and muscle wasting checked, by the subcutaneous injection of salts of the calcium group, there is still doubt as to whether the mode of treatment was such as to insure a sufficient concentration of ions in the atrophying muscles. It is possible that the intravenous injection of larger quantities of the salts would have been more effective. Experiments to this end have been undertaken by the senior author.

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LABORATORY METHODS

A CONVENIENT, AUTOMATIC APPARATUS FOR EXPERIMENTS WITH SURVIVING ORGANS*

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THE arrangements commonly used for studying the activity of excised organs in physiology and pharmacology are cumbersome, inefficient, and inconvenient. Frequently they lack convenient devices for proper control of temperature, washing the organ, etc. Anyone who has worked extensively with excised organs realizes the importance of controlling these factors promptly and efficiently in order that true and accurate results may be obtained. Therefore, it seemed desirable to construct an apparatus which would help to overcome these disadvantages and difficulties. The apparatus here described, it is believed, answers the requirements along these lines. The heating mechanism of this apparatus was suggested by a similar device used in a heart perfusion apparatus described by Fröhlich and Pollak.†

Briefly, the apparatus consists of an electrically heated brass chamber with inflow and outflow for the physiologic solution, and contains a small brass tube for admission of oxygen or air, a thermometer and an adapted heart lever for recording movements of the excised organ on a kymograph. The chamber is clamped on an iron support to which is screwed a pine board for attachment of a three contact switch and a resistance lamp, which maintains the temperature at a constant level of 38° C. In addition there is a glass leveling bulb for the physiologic (Tyrode, Locke or Ringer) solution, which is conducted to the inflow of the chamber by rubber tubing. The arrangements for heating, removal and replacement of the solution are practically automatic, requiring a minimum of time (about 1½ minutes) and effort for manipulation.

In detail, the apparatus consists of the following parts and their dimensions. *Chamber for excised organ;* this consists of a brass cylinder with tapering end into which is screwed a medium sized, metal stopcock. The diameter of the cylindrical portion is 3.8 cm. (1½ inches) and the length, 6.8 cm. (3½ inches). Its capacity is 50 c.c. to the mouth of the outflow tube soldered into and near the upper edge of the chamber. The outflow tube also serves as a handle for clamping the chamber on the iron support and consists of brass tubing 1 cm. in diameter and 14 cm. long. The wiring and insulation of the chamber are made as follows: First, the cylindrical portion of the chamber is covered with a layer of mica. Then, 48 feet of 27 guage Advance wire are wound in a single layer over the mica, and on top of this is laid a thin sheet of asbestos. The entire covering is held in place by 2 brass bands, one at the top and one near the bottom of the cylinder. A post in each band serves for attaching wires from the resistance and main current. The aeration tube within the chamber is made from a small brass tube of 3 mm.

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†Fröhlich and Pollak: Arch. f. d. ges. Physiol., 1920, 184: 14.

outside diameter and 1 mm. bore, and extends to the bottom. It is soldered to the inside wall of the chamber. The upper end of it bends over the edge of the chamber for attachment of rubber tubing conveying oxygen or air under pressure. *Lever*: this consists of an ordinary heart lever of the Harvard type with a small iron rod (10 cm. long), bent to a right angle at its distal end, the straight end being inserted in a socket in the frame underneath the axle. The lever is held in place by a small adjustable clamp on the iron support. The angle rod which is used for fastening the excised organ can be readily withdrawn from its socket and the lever used for other purposes. *Thermometer*: a short thermometer (about 15 cm. in length) is used and supported by 2 brass rings soldered inside

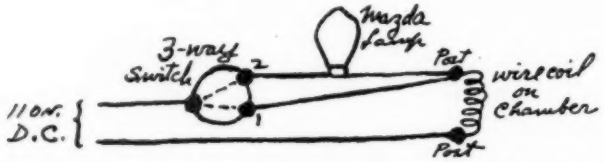

Fig. 1.—Diagram of Connections in Switch and for Resistance. At *1* the full 110 v. D. C. current is obtained; at *2*, resistance, which maintains the temperature of the chamber at 38° C.

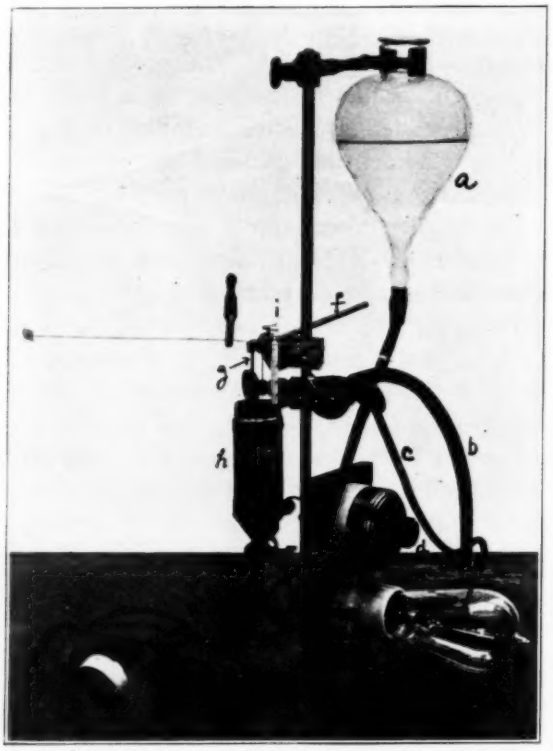

Fig. 2.—Photograph of the entire apparatus set up for use. (*a*) 1 L. Levelling bulb; (*b*) rubber tubing attached to outflow tube; (*c*) rubber tubing attached to aeration tube for oxygen or air; (d) 3-way switch; (*e*) 40-watt Mazda Lamp; (*f*) heart lever with (*g*) L-shaped rod for suspension of excised organ in (*h*) chamber; (*i*) thermometer, and (*j*) wires from socket and lamp to chamber.

the chamber. *Iron support*: an ordinary support about 45 cm. (18 inches) tall with a broad base suffices. *Glass bulb for the physiological solution:* this consists of a levelling bulb of 1 Liter capacity. *Resistance*: for this a 40 Watt Mazda lamp is used and attached together with a 3-contact switch to a small pine-board at the lower end of the support. A numbered 3-way switch is most convenient, giving the full 110 v. current at No. 1 and resistance at No. 2. A diagrammatic sketch of the wiring for the switch and resistance is presented in Fig. 1. Fig. 2 shows a photograph of the entire apparatus set up for use.

Method of Operation: Having filled the chamber with the physiological solution (Tyrode, Locke or Ringer), the switch is turned to No. 1, which gives the full current of 110 volts. The temperature in the chamber rises rapidly (in about 22 seconds) to 31° C. When it reaches 31° C. the switch is pressed to the next contact, i.e., No. 2. This connects with the resistance lamp. The temperature now rises more slowly, requiring about 1½ minutes to reach 38° C., and it remains at this level permanently. Variations in the temperature in the chamber may occur from variations in the electric current, and variable aeration. Aeration should be conducted at the rate of about 1 bubble per second. The strip of excised organ or tissue is introduced either before or after the temperature is raised. Removal of the solution is accomplished automatically by opening the stopcock at the bottom of the chamber and allowing it to flow through the handle, which serves as the outflow tube. The time necessary for removal of 50 c.c., i.e., one complete washing of the chamber, is about 20 seconds. In order to prevent toxicity effects from the chamber, when corrosion occurs, it may be nickelled or the inside coated with paraffin of high melting point (50° C.).

ADVANTAGES

The apparatus is simple, compact, easily transferable, permanent and inexpensive. It has been found very convenient for research and teaching. It has the following important advantages for mammalian tissues: warming of the organ or tissue is rapidly accomplished, and this facilitates the initiation and maintenance of functional activity and recovery from previous effects; overheating is practically impossible, and the organ is not exposed to the atmosphere during washing, or removal of solutions. The apparatus can also be used for amphibian tissues.

Thanks are due to Mr. Morris Dan of Cleveland for mechanical assistance in construction of the apparatus.

A CONVENIENT FORM OF TEST TUBE RACK*

By Sterne Morse, M.D., New York City

TEST tube racks in use have one practical disadvantage which is to a large extent overcome in the rack here figured. This is the necessity of removing a tube under examination in order that the whole of its contents, sediments, as well as supernatant liquids can be thoroughly examined. The type of rack here

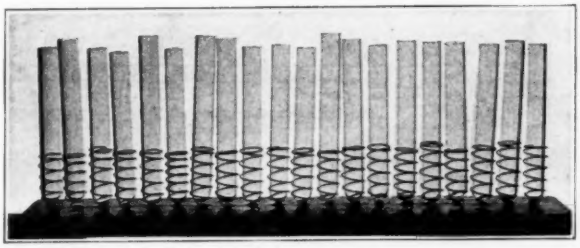

Fig. 1.

figured is so constructed, that every part including the bottom of the tube can be thoroughly inspected without touching the tube. It also has the merit of lightness, cheapness and ruggedness.†

*Accepted for publication, April 27, 1922.

†This rack in several different types, can be obtained from Eimer and Amend, 218 Third Ave., New York City.

WASSERMANN TEST TUBE AND PIPETTE WASHER*

By C. E. Swanbeck, M.D., Cleveland, Ohio

THE apparatus shown in the diagram was made from two nickel-plated brass tubes, which previously had served as towel racks. Ten small holes were drilled in each tube. The distance between each hole was made to correspond to the distance between the test tubes in the Wassermann rack. A five-

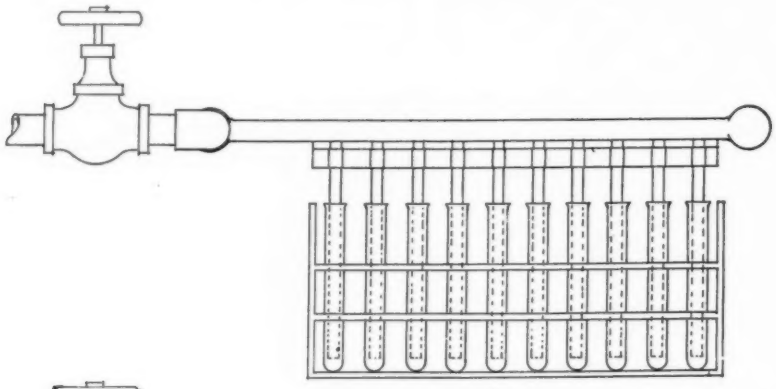

Fig. 1.

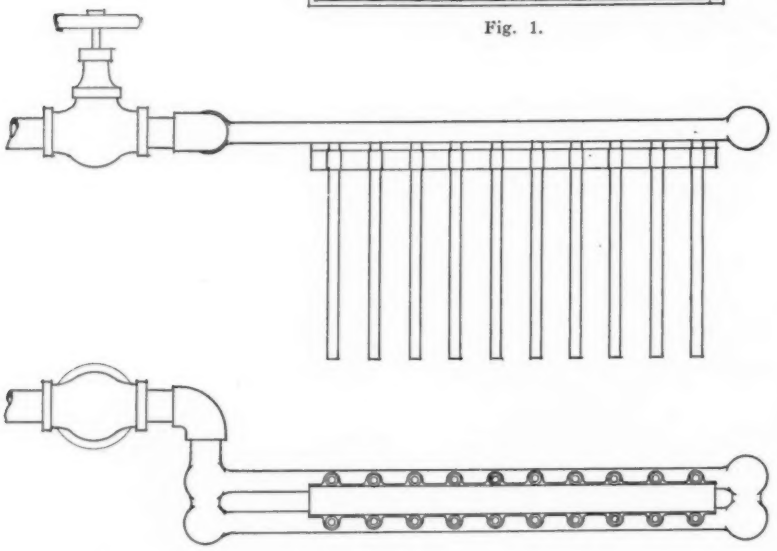

Fig. 2.

inch length of copper tubing with an outside diameter of ¼ inch was now inserted in each hole and soldered in place. The two brass tubes containing the copper tubing were now united at each end with pipe couplings and held apart so that the copper tubes were in the same relationship to each other as the holes in the Wassermann racks. At one end was inserted a pipe fitting to

*From the Laboratories of Mt. Sinai Hospital, Cleveland, Ohio.
Received for publication, Dec. 12, 1921.

be attached to the general water supply pipe in the sink. In order to reinforce the copper tubing a wooden strip was inserted between the two rows of copper tubing and a narrow strip of copper tacked on in such a way that it held the copper tubing against the wooden strip.

When a rack containing Wassermann tubes is now attached to the above apparatus and the water turned on the tubes are thoroughly cleaned in ten minutes.

This outfit was devised because on testing some of our Wassermann tubes with benzidine a positive test for blood was occasionally found. When a large number of tubes are left to an orderly to wash, the washing is often super-

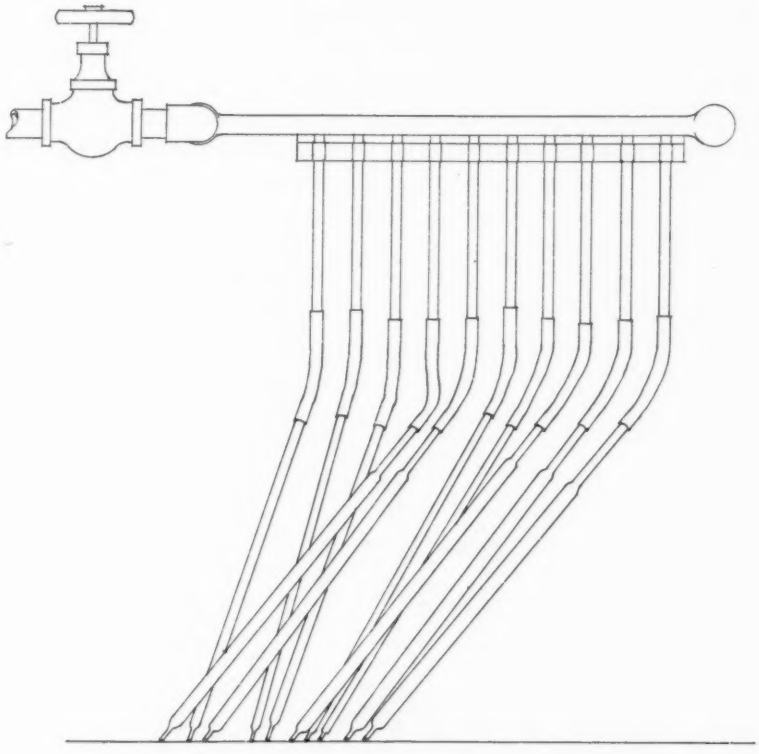

Fig. 3.

ficially done. With this outfit, the orderly places the rack of tubes as he receives them up to the washing apparatus and turns on the water supply, leaving the rack to wash for 15 minutes. Then the tubes are placed in distilled water to soak before drying and sterilizing.

By attaching short strips of rubber tubing to the ends of the copper tubing one can insert the serological pipettes into the rubber tubing and wash these in a similar manner. Twenty pipettes can be washed at one time on each washing outfit.

The advantages of this outfit are, (1) that the tubes and pipette are thoroughly clean, (2) there is considerable saving of time, (3) the washing can be left to an orderly.

A CONVENIENT APPARATUS FOR SIMULTANEOUS DETERMINATION OF TOTAL NONPROTEIN AND UREA NITROGEN, AND FOR PREVENTION OF BUMPING OF FILTRATE DURING BOILING*

By John Walker Moore, B.S., M.D., and Louise Jones, Louisville, Ky.

THE estimation of total nonprotein and urea nitrogen in the blood has become nation-wide in the study of diseased conditions, since the publication in 1919 of Folin's "New Methods of determination of nonprotein nitrogenous products of the blood." The popularity of the methods as are recommended by Folin is embraced in the facts that they are less time consuming than methods heretofore employed, and that with a small amount of blood, a single filtrate

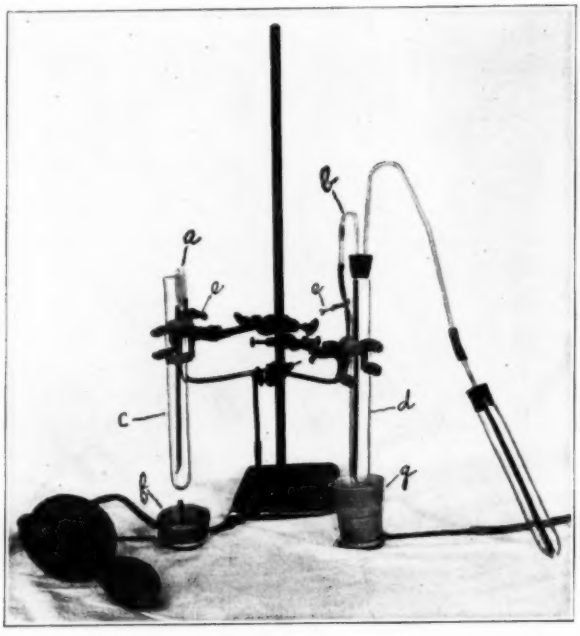

Fig. 1.

can be obtained in which the total nonprotein nitrogen and the various nonprotein nitrogenous products can be carried out with considerable accuracy. The technic of the methods recommended for the determination of the total nonprotein and urea nitrogen, simple though it seems, is not without a serious drawback, in that, bumping of the filtrate when heat is applied to the test tube is prone to occur, even though all precautions that are recommended have been rigidly

*From the Medical Research Department University of Louisville.
Received for publication, February 6, 1922.

observed. Particularly is this true in the distillation method recommended for urea determination. Here a single bumping usually invalidates the test, either by the filtrate going over into the receiving tube, or the acid of the receiving tube being sucked back into the filtrate.

Many ingenious procedures and contrivances have been advised from time to time, no one of which embraces simplicity and brevity and at the same time is absolutely successful.

The apparatus herein described, fulfills the requirement heretofore set forth, and has the advantage that the estimation of total nonprotein and urea nitrogen can be carried out successfully by one person, in the time allotted for a nonprotein nitrogen determination in the original method. The apparatus as shown in the photograph, is essentially that as recommended by Folin, with the addition that glass tubes (*a* and *b*) with deflected capillary points are inserted into test tubes. It will be noticed that these tubes are connected with rubber to the curved arms of a Y-shaped brass tube, whereas the straight arm of the Y is connected to a protected rubber bag for inflation. In the set up, test tube (*c*) is used for the total nonprotein nitrogen, and test tube (*d*) for urea nitrogen determinations. With filtrate in tubes, the rubber bag is inflated and a constant current of air is allowed to escape through capillary tip. A vigorous air current is not essential, it is therefore necessary to regulate air stream by use of rubber screw clamps (*e*). The flame is applied to each tube by means of a micro-burner, and the digestion and distillation of the filtrates are carried out simultaneously in the time and manner recommended by Folin. During the process of digestion, it is not desirable to continue the air current in the nonprotein tube after the filtrate is about half evaporated, for at this stage of the digestion, bumping is not annoying, whereas if the current is allowed to continue, solidification will occur before oxidation is complete. It is not desirable, therefore, to have tip of tubing in test tube (*c*) extending farther than 1.5 cm. of bottom of tube. On the other hand, in the distillation method of determining the urea nitrogen, the capillary tip extends to within 0.5 cm. of bottom, as shown in test tube (*d*), and the air current allowed to continue throughout the test. This procedure not only promotes smooth boiling, but at the same time exerts a pressure which acts as a safeguard against suction that might take place during the determination. Using the method herein described, all annoying bumping is absolutely prevented. The flame can be cut down or increased at any time during the process of digestion or distillation without fear of disastrous results.

We have found the ordinary collapsible drinking cup a convenient and adequate protection for the flame of the microburner. In (*f*) the cup is collapsed, whereas in (*g*) the cup is opened.

CHANGES IN NUTRIENT AGAR DUE TO CLARIFYING WITH EGG*

By Luther Thompson, Urbana, Ill.

WHILE working with lead subacetate agar it was noticed that the results of a previous period could not be duplicated, although the conditions in both instances seemed to be identical. By a process of elimination the only factor not common to both sets of experiments was determined to be the method

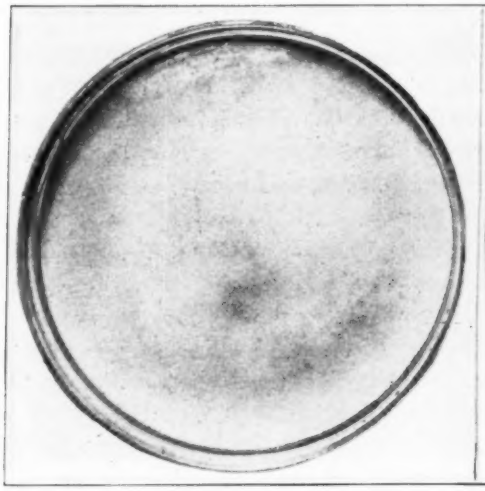

Fig. 1.—Centrifuged agar 24 hrs. incubation.

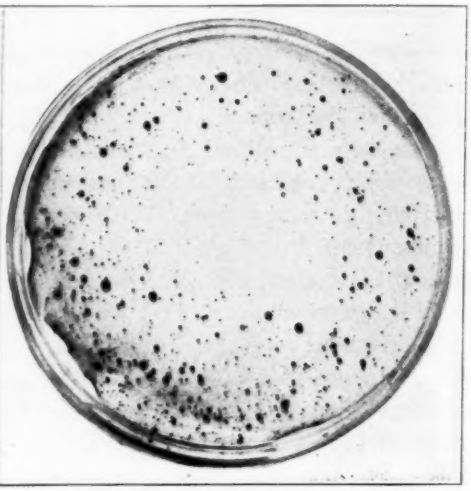

Fig. 2.—Agar cleared with whole egg 24 hrs. incubation.

Bacterium typhosum.

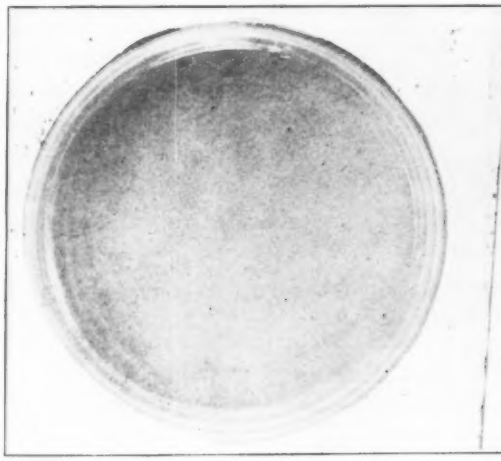

Fig. 3.—Centrifuged agar—24 hrs. incubation.

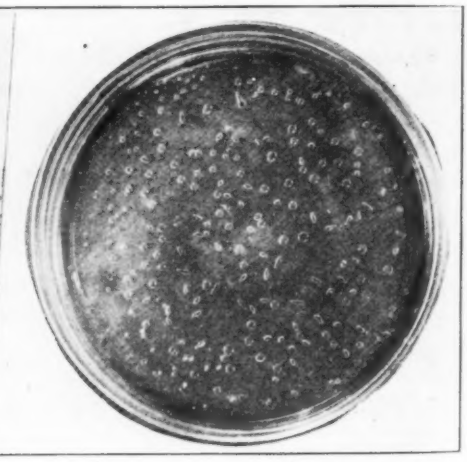

Fig. 4.—Agar cleared with egg white, 24 hrs. incubation.

Bacterium communis.

*From the Department of Bacteriology, University of Illinois.
Received for publication, February 13, 1922.

of clearing the agar, the first being an agar cleared by the addition of egg, and the second an agar cleared by centrifuging.

This suggested an inquiry concerning the effect upon agar of using egg or egg white as a clarifying agent. Accordingly two liters of agar were prepared as nearly as possible in the same manner except that one was clarified by the addition of two whole eggs, and the other by centrifuging. The reactions of both were made neutral to Bromthymol Blue and sterilized in the autoclave for 15 minutes at 15 pounds. To 100 c.c. of each agar was added 2 c.c. of a 2 per cent solution of lead subacetate. Tubes of each were then inoculated from an agar slant of Bact. typhosum and plates were made. Figures 1 and 2 show the results for the hydrogen sulphide test in the two kinds of media. This would indicate that egg adds to the agar certain protein compounds in which the sulphur is readily available, as for example cystin which is found in egg white.[1] Further tests made on agar cleared with egg white and egg yolk respectively, showed that such sulphur compounds may be derived from either the white or yolk of egg, and moreover, that when the yolk is fractioned to obtain hematogen and vitellin, these latter substances will add sulphur compounds to agar which are not coagulated by heat. In all cases where a part of the egg is used, lesser amounts of sulphur are added, which is further proof that the compounds containing it are not restricted to any one of the several proteins found in egg.

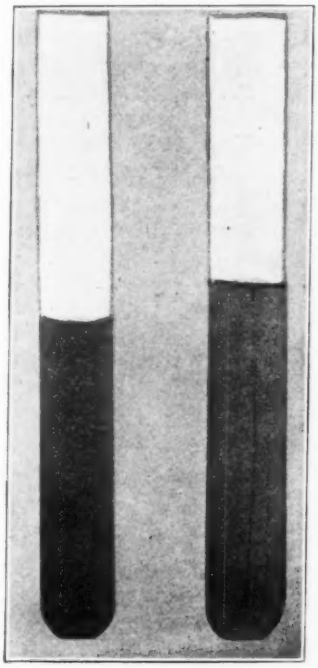

Fig. 5.—Tube at right—agar cleared with egg white. Tube at left—centrifuged agar. Clostridium putrificum—24 hrs. incubation.

The reaction of agar is made alkaline by egg white and acid by egg yolk. Using Bromthymol Blue as indicator, the following reactions were noted in three batches of agar just after clearing:

With egg white .....P_H 7.2
With egg yolk .....P_H 6.4
With whole egg .....P_H 6.8

In each case an equal amount of egg substance was used and the reaction read from color standards.

A fact seemingly of greater significance than those previously mentioned is that of the addition of glucose to agar from the white of egg. Figs. 3 and 4 show the comparison between centrifuged agar and agar cleared with egg white. Both were inoculated with Bact. communis, but the one made with the egg agar shows numerous gas bubbles throughout, thus showing the presence of a fermentable substance. This finding of sugar in such decided quantity in the agar suggests that such a medium might be more favorable for anaerobic

culturing than centrifuged agar, and such appears to be the case. Fig. 5 shows two deep stab cultures of Clost. putrificum. After 24 hours' incubation the egg agar shows an abundant growth along the inoculation, while the other shows but little growth. The reactions of the two media were practically the same. Other tests made in the same way confirmed the first result. The egg agar also gives somewhat larger surface colonies than the centrifuged agar in 18-24 hours on petri dish cultures with B. communis and Bact. typhosum.

The Committee on the Descriptive Chart[6] has permitted the use of egg white as an alternative method of clarifying gelatine. If the results given above are true this would produce a medium considerably different from one obtained by filtering or centrifuging.

CONCLUSIONS

In making a standard agar the method of clarifying should be specified. An agar which is egg-cleared is a special, or egg agar because:

1. It contains sulphur compounds derived from the egg.
2. The reaction of the agar is influenced.
3. A fermentable substance is added, presumably glucose from egg white, which in both anaerobic cultures and on aerobic plates affects the rate of growth of certain bacteria.

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AN EASY METHOD OF OBTAINING SAMPLES OF URINE FROM THE MALE DOG*

By Frank C. Mann, M.D., Rochester, Minn.

TWO methods are usually employed for obtaining urine from the dog; one by keeping the animal in some form of metabolism cage where the excretions are caught, and the other by catheterization. The former method requires a specially constructed cage and there is always the possibility of contamination of the urine with fecal material. The latter method is only applicable to the female as routine catheterization is not feasible in the male dog unless urethrotomy has been performed. Repeated catheterization in either the female or male dog after urethrotomy is often followed by cystitis even though good technic is maintained. A satisfactory procedure is as follows:

The animal is kept in a cage since there is then a greater tendency to al-

*From the Division of Experimental Surgery and Pathology, The Mayo Foundation, Rochester, Minn.
Received for publication, March 8, 1922.

low the urine to collect in the bladder. A Hoffman screw clamp is placed on the prepuce in a manner that will just prevent the escape of urine. To obtain the specimen in the average animal remove him from the cage and give him his freedom, preferably in the run where he takes his daily exercise. Usually in a very short time he will urinate into the sheath of the penis. By gently loosening the screw clamp the urine can be collected in a container. Specimens can thus be obtained at one to two-hour intervals throughout the day.

The method is not applicable if the total amount of urine is desired at a definite time, as the bladder may not be completely emptied, and also bacteriologic studies of the urine cannot be made. If specimens only are desired, the procedure requires less time, less assistance, and less inconvenience to the animal than catheterization. By this method it is possible to obtain specimens of urine from a dozen male dogs in half an hour's time.

If it is desired to drain the bladder constantly, a ureteral catheter which is easily passed in the male dog, is inserted so that one end lies in the bladder and the other in the cavity of the sheath. The screw clamp is then applied to the prepuce. Urine will drain from the bladder through the ureteral catheter into the cavity of the sheath practically as fast as it is secreted, from which it can be drained every hour or two.

The average animal usually does not pay the slightest attention to the clamp and if care is exercised in its adjustment it may be left in position for many hours without inconvenience to the animal. It should be emphasized that the procedure does not cause pain.

A BOX FOR HANDLING SMALL ANIMALS IN THE LABORATORY*

By Linwood D. Keyser,† M.D., Rochester, Minn.

FOR the past two years in the Laboratory of Surgical Research of the Mayo Foundation we have used a simple box for controlling small laboratory animals such as rabbits and cats, so that tubation, feeding, and intravenous injections into ear veins can be carried out easily by one person.

The box is oblong, its dimensions being 42.5 by 22.5 by 22.5 cm. It is divided into two segments, which are hinged (Fig. 1). In each end is a circular aperture, one 6 cm. in diameter, and the other of 5.5 cm. in diameter. When the box is open the apertures are divided into semicircles, two in the upper and two in the lower half of the box. The two apertures offer a range of size suitable for the heads of most rabbits.

The animal (rabbit or cat), is placed in the box, and the box closed with the head of the animal protruding through the aperture, pillory fashion. A hinge holds the box closed (Fig. 2). The animal's head is held relatively fixed and the investigator is protected from scratching. For esophageal intubation

*Received for publication, April 22, 1922.
†Fellow in Pathology, Mayo Foundation.

a No. 10 French soft rubber catheter is introduced through a small opening in a wooden tongue depressor which has been pried between the teeth of the animal as a mouth gag. The catheter is passed into the esophagus by touch and to the stomach. Fluids or suspensions may thus be introduced into the stomach as shown in Fig. 2.

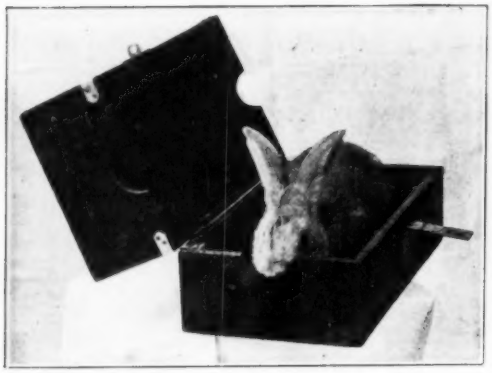

Fig. 1.—Box open showing relation of parts.

Fig. 2.—The box in use showing manner of introducing fluid through tube into the stomach. The animal suffers no pain.

For the intravenous injection of bacteria, drugs, and so forth, the ear is caught at its tip and the head is gently but firmly pulled forward away from the box. The hypodermic needle is then introduced into the marginal vein and the injection made.

Animals handled in this manner are subjected to a minimum amount of discomfort.

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EDITORIALS

Internal Secretion

ENDOCRINOLOGY has reached the apogee of its popularity as a vehicle for extravagant hypotheses. At a time when the ductless glands are being referred to in a semiscientific publication as the "glands regulating personality," when periodicals and three volume treatises are devoted almost exclusively to the literature of endocrinology, when the subcutaneous injection of extracts from ciliary bodies is recommended for the treatment of iritis and of extracts of heart muscle for myocardial disease, when one reads that the internal secretory glands "already unfold before the astonished gaze of the seeing eye a land of promise beside which the vast territories conquered by Lister and Pasteur are destined to pale into honorable insignificance," at such a time one is refreshed and the mind becomes clearer on reading a vigorous protest from the pen of a physiologist who has been a recognized leader in the study of the internal secretions. Swale Vincent,

professor of physiology in the University of London, in an address before the Royal College of Surgeons has made a critical examination of current views on internal secretion. (Lancet, London, August 12, 1922.)

Theophile de Borden in 1775 first promulgated the theory that each organ in the body secretes into the system a special substance which acts on the other tissues of the body. This held for all organs no matter what their nature. J. Müller in 1838 discussed those glands without ducts and believed that they exerted a certain plastic influence on the fluids circulating through them. Berthold of Göttingen first demonstrated experimentally the existence of an internal secretion in 1849, by removing testes from young cockerels and transplanting them to the surface of the intestine. The doctrine of internal secretion as it is generally taught today was founded by Claude Bernard and Brown-Séquard.

The study of internal secretion has consisted chiefly in observing the effects of injection of organ extracts into experimental animals. The reactions observed are pharmacodynamic only and do not necessarily express the physiologic activity of the organ under consideration. The existence of these active substances found experimentally does not necessarily prove that the organs from which the extracts were made possess an internal secretion or that that secretion acts in the economy in the same manner in which it acts in the laboratory. Thus it has been found that an extract from the posterior lobe of the pituitary body of a bull will cause contraction of the uterus of a female guinea pig. It is quite impossible to visualize such a function existing in the animal from which the extract was made. The observations are, to be sure, of great pharmacologic interest but it is not until after many other lines of research have been followed that one would be justified in drawing conclusions as to the presence and action of an internal secretion.

Early investigators in the field of endocrinology found that an extract made from the medulla of the adrenal bodies, when injected into animals, caused certain characteristic reactions, chiefly of a pressor nature and affecting principally the heart, blood vessels and other types of muscle. The same was found to be true of extracts from that portion of the pituitary body which is of nervous origin. As a result of these and similar observations an extravagant theory was built up to the effect that many or most of the bodily functions are controlled by the balancing of pressor substances secreted from these two sources and certain antagonistic or depressor internal secretions formed elsewhere in the body. In spite of all the work that has been done in recent years on the physiology of the adrenal bodies we know as yet little concerning their function. The function of the cortex, the larger portion of the gland, is unknown. There can be little doubt that the medullary portion actually secretes small amounts of epinephrin into the circulation. The importance of this substance to the general physiology is by no means as certain. Vincent goes so far as to state that there is little or no evidence that the medulla of the adrenal bodies is of any importance in the normal economy of the animal. He believes that notwithstanding the views of Cannon it is doubtful whether the adrenalin output is at all affected by emotional excitement.

The prevailing hypothesis of thyroid function is based on sound chemical and pharmacologic research and on more convincing clinical evidence than holds for the adrenal gland. Thyroxin, isolated by Kendall, acts through the blood stream as a catalyzer for the production of energy within the tissues. Thyroxin causes no change in the chemical reactions within the body but merely increases the rate of reaction. According to Kendall the thyroid gland has been added to the animal organism to permit a greater flexibility of energy output. Altered function of the thyroid gland is fairly easily recognized and treatment is fairly successful whether the alteration be in the nature of hyper-function or hypo-function. However the diagnosis of hyper-function or hypo-function is often made on insufficient evidence and when the actual pathology is elsewhere.

From knowledge of the pharmacology of pituitary extracts, and from a study of diseases associated with abnormalities of that organ, we have some fairly definite information regarding its activity. The problem is however complicated by the fact that in all probability pituitary function is interrelated with the function of certain other endocrine glands. Moreover some of the recent work indicates that many or all of the abnormal findings usually attributed to a lesion of the pituitary body are in reality the result of damage to the base of the brain in the region of the sella turcica and not to disease of the hypophysis itself. In clinical work there is a well recognized tendency to class all growth anomalies as pituitary in origin and the term "dyspituitarism" has acquired a diagnostic popularity which the facts do not warrant.

Recent work indicates that the parathyroids control muscle tone by keeping down the amount of some toxic substance, possibly guanidin, in the circulating blood. This is accomplished either by checking its production or by bringing about its conversion into creatin. There is a tendency to classify all "tetanic" disorders as due to lesions of the parathyroid glands.

According to Vincent our knowledge of the internal secretion from the pancreas and the gonads is based chiefly on clinical rather than on experimental observations. However, the epochal work of Banting and Best reported in this Journal in February and May, 1922, in which they have a pancreatic enzyme capable of splitting sugar and diminishing glycosuria and hyperglycemia, promises to be one of the most valuable, recent discoveries. The value of such an extract in therapeusis will easily equal that of thyroid extract.

We know nothing definite concerning the function of the thymus.

In spite of paucity of actual knowledge of the function of the internal secretory glands, the pharmaceutical market is virtually flooded with preparations made from extracts of the various body organs. It seems logical to conclude that these substances would not be manufactured in such abundance if they did not find a ready market among practicing physicians. Practically all are without doubt quite inert when taken by mouth. The active substance from the thyroid gland is possibly the only one that exerts any influence when so administered.

Organotherapy is as old as is the art of medicine, and the superstition

which impelled the savage to devour the heart of the lion so that he might acquire the courage of that animal is the same superstition which will impel a physician to prescribe prostatic extract for disease of the prostate gland or senility or for impotence. Celsus and Dioscorides recommended the use of various animal organs for the relief of symptoms considered to be due to impaired function of those same organs in man. Cushing suggests that "The Lewis Carroll of today would have Alice nibble from a pituitary mushroom in her left hand, and a lutein one in her right, and presto! she is any height desired."

Vincent does not deny the undoubted importance of endocrine function in physiology, but he voices his protest at the indiscriminate and uncritical application or misapplication of the meagre knowledge at our disposal. "There is indeed serious danger that the nucleus of sound knowledge which justifies a belief in the doctrine of internal secretion may be jeopardized by a too drastic destructive criticism, in the same kind of way that, when phrenology was exploded by Flourens in 1822, brain physiology was so seriously injured in the fray as not to recover for close upon half a century. One hears cynically inclined onlookers and medical scoffers suggesting at the present time that we should encourage the vitamins in order to keep down the plague of endocrines, and prophesying that sooner or later some new fad will be welcomed whose function it will be to relieve the strain of vitaminology."

—*W. T. V.*

Activities of the Medical Section of the League of Nations

WHATEVER may be the condition of other sections of the League of Nations, the Medical Section evidently is far from being moribund. In December, 1921, a committee of this Section met in London for the purpose of taking steps for the standardization of antitoxins and other biologic products. So far as diphtheria is concerned, the units in different countries are not materially unlike, but in the case of tetanus there are great differences, and it is proposed that committees with members from various countries try to establish standards for these agents. A proposition has been made that sailors be treated free of charge in any country they might be when needing treatment. This is especially desired insofar as treatment for syphilis is concerned. Another thing that the Medical Section of the League of Nations is attempting to do is to standardize the Wassermann Test; also to standardize or define arsenical preparations used in the treatment of syphilis. These certainly are commendable undertakings.

In May, 1922, the Medical Section of the League of Nations held a meeting in Paris and discussed some most important health and medical subjects. In 1920 an international congress was called in Paris for the purpose of establishing an international list of the causes of death. This work has never been completed and the list has not been published. At this May meeting in Paris the Medical Section of the League of Nations took

steps to complete this list of causes of death and to secure immediate publication. At the same meeting the opium question was discussed from all angles. An Englishman from India held that in that country opium is taken as tea or tobacco is taken in other countries. It is a stimulant for workers engaged in arduous labor, for soldiers who have to make long marches, and even for animals. He admitted that its employment might have injurious effects on the young, but that it is not harmful after forty years of age when the vital energy is on the decline. Unlike cocain, it does not tend to increase crime. Insurance companies do not distinguish between those who take and those who do not take opium. In thousands of villages opium takes the place of medicines for all kinds of sickness, and it also plays a part in certain religious practices with which it would be dangerous to interfere. This seems queer, and it is rather difficult for us to believe. It is highly proper that the Medical Section of the League of Nations refused to endorse this statement and decided on further investigation.

At the Paris meeting it was proposed that students of epidemiology in one country be invited into other countries and given opportunity therein to continue their studies. This would be of the greatest value to epidemiology. This is a subject which needs to be studied in its world-wide aspects and it would be of great advantage if French or English students of epidemiology could go into Russia and study, together with the native epidemiologists, the diseases now prevailing in that country. The Section passed the following resolution: "The Health Committee, taking note of the proposal regarding an interchange of sanitary staff between different countries interested in its being carried into effect, esteeming it of the greatest utility for sanitary officers to become acquainted with practical solutions of public health problems as well as with the organization of public health services in various countries, recommends that the necessary steps should be taken by approaching the governments interested in the matter in order to carry into effect this interchange, at least as a provisional and experimental measure."

It was deemed desirable that the appointment of a committee consisting of experts nominated by the Mandatory Powers in Africa should collect information available regarding the prevalence of sleeping sickness and tuberculosis among the native population of Equatorial Africa and the measures recommended to prevent the extension of these diseases in this region should be determined.

Steps were taken for the strengthening of international arrangements for the prevention of epidemic diseases in certain areas of the Near East and in connection with the Mecca pilgrimages. It seems that nothing in an international way has been done in this matter since 1912. Pilgrimages in the near future may scatter disease far and wide over the three continents of the Eastern Hemisphere and possibly may extend the distribution of these viruses to the Western Hemisphere. It is pleasing to note that, notwithstanding our country is not a member of the League of Nations, the Medical Section of that body has invited Dr. Josephine Baker, Director of the Bureau of Child Hygiene in New York City, to become a member and

to participate in their deliberations. This is a recognition of the splendid work Dr. Baker has done in New York, and we are sure that the medical profession throughout the United States will agree that she well deserves this distinction. The work of the Epidemic Commission of the League is the first instance of international effort in public health. It differs fundamentally from the activities of various relief organizations, inasmuch as it is supported entirely by the governments concerned and is in no sense a charitable organization. The Epidemic Commission has not undertaken field work of its own, but has acted through the public health administration of the countries concerned. It has declined to have any relation with the relief organizations except through the intermediary of its government. It bases its work on the necessity of strengthening the public health and sanitary organization in the country where it is needed. Relief organizations as a rule are interested in the immediate alleviation of sufferings, while the Epidemic Commission looks forward to more permanent results. The epidemic situation in Europe, especially in Russia, will continue to be serious for years to come and it is certain that the Epidemic Commission of the League of Nations will have to function for a long time. If the developments in the scheme of the economic reconstruction of Russia should allow of sufficient funds being collected through the government the Epidemic Commission should be the medium for putting into effect any such scheme. It was suggested at the meeting that steps be taken to facilitate the methods of securing cheap supplies of quinin and arsphenamine in all the countries of the world. —*V. C. V.*

Cultivation of the Gonococcus

ERICKSON and Albert[1] have tested out the various media on which the gonococcus has been grown and conclude that testicular blood agar with a reaction of P_H 7.4-7.8 is the most favorable medium for the isolation and subsequent cultivation of this organism. Beef testicle, from which all connective tissue has been removed, is put through a meat grinder and infused overnight with twice its weight of distilled water. The next morning the mixture is heated to 50° C., allowed to stand for one hour, and then brought to the boiling point. After standing for another hour in order to allow the solid particles to settle, the supernatant fluid is decanted and used for the preparation of culture media. To this infusion, 2 per cent peptone, .5 per cent glucose, .3 per cent monobasic sodium phosphate, and 2.5 per cent powdered agar are added. This mixture is heated until the agar is dissolved, then treated with phenol red and the reaction adjusted to the above-given figures. The medium may then be tubed and autoclaved for twenty minutes at fifteen pounds. The titration may be checked after sterilization. While the contents of the tubes are still liquid, human blood is added in the proportion of .5 to 2.5 per cent. If human blood is not at hand defibrinated rabbits' blood (1 to 5 per cent) may be employed. —*V. C. V.*

[1]Jour. Infect. Dis., 1922, xxx, 268.

INDEX TO VOLUME VII

AUTHORS INDEX

In this index following the author's name the full title of the subject is given as it appears in the Journal. Editorials are also included in the list and are indicated by (*E*).

A

ARMOUR, M. E. (*See* Macleod and Armour), 635
ASCHAM, LEAH. (*See* Smith, Deuel, Ascham, and Seibert), 473
ATKINSON, H. V. The treatment of acute phosphorus poisoning, 148

B

BAILEY, PERCIVAL. (*See* Stevens and Bailey), 746
BAILEY, WM. H. The laboratory specialist as a clinical consultant, 410
BANTING, F. G., AND BEST, C. H. Pancreatic extracts, 464
—AND—. The internal secretion of the pancreas, 251
BARNES, ARLIE R. (*See* Giordano and Barnes), 538
BAUMAN, L., AND KEELER, L. M. Suggestions for the determination of uric acid in blood, 551
BAUMANN, EMIL J., AND ISAACSON, RAE L. An adaptation of the Folin and Hu blood sugar method applicable to small amounts of blood, 357
BEST, C. H. (*See* Banting and Best), 251, 464
BIRCHER, M. E. Clinical diagnosis by the aid of viscosimetry of the blood and the serum with special reference to the viscosimeter of W. R. Hess, 134
—. II. The value of the refracto-viscosimetric properties of the blood serum in cancer, 660
—. III. The value of the refracto-viscosimetric properties of the blood serum in cases of tuberculosis, 733
BIXBY, B. J. (*See* De Niord and Bixby, 573
BLISS, A. RICHARD, JR. An experimental investigation of the pharmacologic activity of drug store samples of infusion of digitalis, U. S. P. IX, 225
BLOEDORN, W. A., AND HOUGHTON, J. E. The role of hexamethylenamina in the production of hematuria, 514
BROWN, J. HOWARD. A method for the separation of spore-bearing anaerobes from other spore-bearing bacteria, 692
BURTON-OPITZ, R. A new type of recording spirometer, 681
BUTSCH, J. L., AND O'BRIEN, CLAIRE M. Devices to eliminate certain objectionable factors in the analysis of gastric content, 431

C

CARTER, CHAS. F. A simple method of counting the cells in cerebrospinal fluid, 555
CHRISTIAN, THOMAS B. Chair for performing spinal punctures, 430
CROLL, HILDA M. (*See* Kast, Croll, and Schmitz), 643

D

DE EDS, FLOYD. (*See* Hanzlik and De Eds), 751
DE NIORD, R. N., AND BIXBY, B. J. Studies in focal infection, 573
DEUEL, HARRY J., JR. (*See* Smith, Deuel Ascham, and Seibert), 473

F

FLORENCE, LAURA. The influence on spore formation of sealing bacterial cultures, 199
FRIEDLANDER, ALFRED. (*See* Jackson, Friedlander, and Lawrence), 311
FUNK, ELMER H., AND WEISS, EDWARD. Mercuric chloride poisoning with recovery. A case report with a note on the urea-concentration test, 233

G

GARDNER, VAUGHN C. (*See* Weiss and Gardner), 229
GILBERT, RUTH, AND MOORE, ANNA C. Widal technic using sterilized cultures, 547
GIORDANO, ALFRED S., AND BARNES, ARLIE R. Studies in postmortem bacteriology: value and importance of cultures made postmortem, 538
GRANT, GARNET B., AND WILSON, ERIC R. A plea for the standardization of the training for laboratory technicians, 562

GUTHRIE, CHARLES. A spirometer for continuously and automatically measuring expired air and sampling for analysis, 421

H

HANZLIK, P. J., AND DE EDS, FLOYD. A convenient, automatic apparatus for experiments with surviving organs, 751

HARTMAN, F. A. Treatment of denervated muscle, (*E*), 433

HELMHOLZ, HENRY F., AND MILLIKIN, FRANCES. The presence of bacteria and formed elements in the urine of rabbits, 589

HIGGINS, JOHN A. (*See* Mielsen and Higgins), 69, 579

HOAGLAND, RALPH. A self-feeder for rats, 687

HOUGHTON, J. E. (*See* Bloedorn and Houghton), 514

I

INLOW, WILLIAM DEP. A technic for the establishment of a permanent pancreatic fistula with the secretion of inactive proteolytic ferment, 86

ISAACS, RAPHAEL. An injection method for aiding in the identification of tapeworm species, 691

ISAACSON, RAE L. (*See* Baumann and Isaacson), 357

J

JACKSON, D. E., FRIEDLANDER, ALFRED, AND LAWRENCE, J. V. An experimental investigation of the pharmacological action of quinidine, 311

JONES, HORRY M. Basal metabolism determination and its technical difficulties, 191

JONES, LOUISE. (*See* Moore and Jones), 756

K

KAST, LUDWIG, CROLL, HILDA M., AND SCHMITZ, HERBERT W. The therapeutic use of germanium dioxide in anemia, 643

KAUFMAN, C. E. (*See* Smith and Kaufman), 619

KEELER, L. M. (*See* Bauman and Keeler), 551

KEHOE, ROBERT A. The activation of an enzyme poisoned by heavy metal salts, 736

KEHRER, J. K. W. (*See* MacCarty and Kehrer), 602

KEYSER, LINWOOD D. A box for handling small animals in the laboratory, 761

KILDUFFE, ROBERT A. A note upon the utilization of the complement-fixation reaction in the diagnosis of tuberculous meningitis, 427

KILLIAN, JOHN A. A note on the blood chlorides in mercuric chloride nephrosis, 129

KOBER, PHILIP ADOLPH. The methyl alcohol in arsphenamine (salvarsan), 168

KOPELOFF, NICHOLAS, AND MORSE, STERNE. What are the atmospheric moisture requirements of bacteria? 555

L

LACY, G. R., AND MURDOCK, CECILIA. Isolation of tetanus bacilli from the cerebrospinal fluid, 100

LAIRD, JOHN L. New laboratory apparatus, 477

LANGSTON, WANN. Glucose tolerance test, 293

LAWRENCE, J. V. (*See* Jackson, Friedlander and Lawrence), 311

LEVINSON, A. The cellular elements of the cerebrospinal fluid, 626

LEVINSON, LOUIS A. The relation of achylia gastrica to chronic focal infections and pernicious anemia, 652

LONG, A. G. (*See* Mendelsohn, McCoy and Long), 208

LORENZ, W. F. An automatic pipetting device, 54

M

MACCARTY, WM. CARPENTER, AND KEHRER, J. K. W. Possible defensive factors in cancer of the rectum. (A study of 102 cases), 602

MACLEOD, J. J. R. The chemical control of the respiratory center, (*E*), 369

—. Vitamins and deficiency diseases, (*E*), 60

—. AND ARMOUR, M. E. The determination of lactic acid in blood, 635

MAHR, ERNEST F. Studies in the serology of syphilis, 1

MANN, F. C. An easy method of obtaining samples of urine from the male dog, 760

—. A technic for making a biliary fistula, 84

MCCASKEY, G. W. Chronic urticaria of five years' duration probably due to chronic acidosis, 534

MCCOY, A. E. (*See* Mendelsohn, McCoy, and Long), 208

MCKINLAY, C. A. Suggested adaptation to the gasometer method of the determination of the basal metabolic rate from carbon-dioxide elimination, 665

MCNEIL, ARCHIBALD. A preliminary report on a method of determining the number of complement binding units in sera giving positive Wassermann reactions, 109

MELTON, HERMAN D. A rapid method of preparing tissues for microscopic examination, 114

MENDELSOHN, I. W., MCCOY, A. E., AND LONG, A. G. Some studies on the elimination of molds from butter, 208

MEISSER, JOHN G. (*See* Rosenow and Meisser), 707

MILLIKIN, FRANCES. (*See* Helmholz and Millikin), 589

MITCHELL, HELEN S. An improved method of caging and feeding mice, 299

MIURA, MASATARO. The effects of thyroid, thyroxin and other iodine compounds upon the acetonitrile test, 349

—. The effects of various foods, food factors and chemical agents upon the resistance of animals to acetonitrile, 267

MORIARTY, MARGARET E. (*See* Talbot and Moriarty), 91

MOORE, ANNA C. (*See* Gilbert and Moore), 547

MOORE, JOHN WALKER, AND JONES, LOUISE. A convenient apparatus for simultaneous determination of total nonprotein and urea nitrogen, and for prevention of bumping of filtrate during boiling, 756

MORSE, STERNE. A convenient form of test tube rack, 753

—. (*See* Kopeloff and Morse), 555

MURDOCK, CECILIA. (*See* Lacy and Murdock), 100

MUSKIN, NATHAN, AND SIEGEL, LOUIS. The making of collodium sacs, 564

MYERS, BURTON A., AND SCHEVKY, MARIAN. The estimation of inorganic phospherus blood plasma by the method of Bell and Doisy, 176

MYERS, C. N. Development of the chemotherapy of organic arsenicals and the related physical phenomena, 17

—. The synthesis of arsphenamine and a study of some of its intermediate derivatives, 151, 215

MYERS, VICTOR C. A new microcolorimeter, 237

N

NEUHOF, S. Some sociological aspects of heart disease, 606

NEUWIRTH, ISAAC, AND KLEINER, ISRAEL S. The blood-sugar content of capillary blood as compared with that of venous blood, 495

NEWCOMB, PHILIP B. An adapted mask for basal metabolism apparatus, 560

NI, TSANG G. Beriberi: some facts and the possible hypothesis, 340

NICHOLIS, ALBERT G. Note on the differential staining of the granules in diphtheria and other bacilli, 180

NIELSEN, CARL, AND HIGGINS, JOHN A. Further observations on the pharmacology of benzyl compounds, 69, 579

O

O'BRIEN, CLAIRE. (*See* Burtsch and O'Brien), 431

OLIVER, SYMMES F. The effect of bile salts in the urine on routine tests for albumin, 743

P

PALMER, GEORGE T. Ventilation, weather, and the common cold, 39

PASKIND, H. A. Some differences in response to atropine in white and colored races, 104

PICKARD, RAWSON J. The clinical value of basal metabolism determination, 669

PRYER, R. W. Etiology of scarlet fever, 592

R

REYNER, C. E. An apparatus for the rapid determination of nitrogen in blood and urine, 53

RHODES, BERNICE. The phenomenon of bacteriophage, 288

RIEGER, JOHN B. A note on the estimation of blood chlorides in tungstic acid filtrates, 166

ROSENBLOOM, JACOB. Blood pressure studies in one hundred and forty cases of diabetes mellitus, 392

ROSENOW, EDWARD C., AND MEISSER, JOHN G. Elective localization of bacteria following various methods of inoculation, and the production of nephritis by devitalization and infection of teeth in dogs, 707

S

SANDERSON, EVERETT S. A note on the sterilization of surgical instruments, 360

SCHEVKY, MARIAN C. (*See* Myers and Schevky), 176

SCHMITZ, HERBERT W. (*See* Kast, Croll, and Schmitz), 643

SCHULTZ, OSCAR T. Removable water-bath tops for paraffin embedding and low temperature evaporation, 689

SEIBERT, FLORENCE B. (*See* Smith, Deuel, Ascham, and Seibert), 473

SHERNDAL, A. E. Arsphenamine—some factors which influence its colloidal properties, 723

SIEGEL, LOUIS. (*See* Muskin and Siegel), 564

SMITH, ARTHUR H., DEUEL, HARRY J. JR., ASCHAM, LEAH, AND SEIBERT, FLORENCE B. Yeast therapy and uric acid excretion, 473

SMITH, G. H., AND KAUFMAN, C. E. The precipitin test in the detection of bacterium diphtheriae, 619

SMITH, MILLARD. A micromodification of the method of Benedict for the quantitative determination of reducing sugar in urine, 364
STEVENS, H. C., AND BAILEY, PERCIVAL. The nature and treatment of muscular atrophy, 746
SWANBECK, C. E. A modification of the Kjeldahl still for distilling large quantities of water, 57
—. Wassermann test tube and pipette washer, 754
SWIFT, DAVID BREWSTER. Rouleaux formation of red cells in various types of disease, 614

T

TALBOT, FRITZ B., AND MORIARTY, MARGARET E. Sources of error in determining the respiratory quotient in the baby respiration apparatus, 91
TAYLOR, N. B. The blood-flow in man as estimated by the colorimetric method of Stewart, 439
THOMAS, J. EARL. A method for producing a spinal dog, 417
THOMAS, J. EARL, AND WHEELON, HOMER. The nervous control of the pyloric sphincter, 375
THOMPSON, LUTHER. Changes in nutrient agar due to clarifying with egg, 757

V

VAN DER HEYDE, H. C. On the determination of small quantities of atropine in blood serum, 280
VAUGHAN, VICTOR C. Activities of the Medical Section of the League of Nations, (*E*), 766
—. Another German cure for tuberculosis, (*E*), 120
—. A research fellowship in bacteriology, (*E*), 503
—. Botulism, (*E*), 116
—. Cod liver oil in rickets, (*E*), 571
—. Cultivation of the genococcus, (*E*), 768
—. Excess protein and mammary secretion, (*E*), 505
—. Further studies on pellagra, (*E*), 631
—. Medical fellowships, (*E*), 502
—. Obituary. G. Sims Woodhead, (*E*), 705
—. The etiology of rickets, (*E*), 701
—. The pathogenicity of B. Botulinus, (*E*), 570
—. What are Russia's medical needs? (*E*), 436
—. Where do the vitamins come from (*E*), 504
VAUGHAN, WARREN T. A new tuberculosis cure, (*E*), 699
—. Arsphenamine and neoarsphenamine dosage, (*E*), 181
—. Eosinophilia in parasitic infections, (*E*), 123
—. Internal secretion, (*E*), 763
—. Liver function, (*E*), 565
—. Medical and health conditions in Soviet Russia, (*E*), 248
—. Newer conceptions concerning shock, (*E*), 498
—. The diabetic diet, (*E*), 694
—. The nature of flutter and fibrillation of the auricle, (*E*), 242

W

WARING, JAMES J. A case of tuberculoma of the spinal cord, 96
WARNOCK, FANNY. The colloidal benzoin reaction of cerebrospinal fluid, 400
WARWICK, MARGARET. Focal embolic glomerulonephritis, 507
WEBB, GERALD B. Intimate exposure and contagion in tuberculosis, (*E*), 306
WEISS, EDWARD, AND GARDNER, VAUGHN C. Chronic nephritis with an unusual degree of nitrogen retention, 229
—. (*See* Funk and Weiss), 233
WELKER, WM. H. An apparatus for the estimation of catalase, 173
WHEELON, HOMER. (*See* Thomas and Wheelon), 375
WHITMAN, R. C. Some time-saving devices for handling paraffin embedded material, 240
WILHELMJ, C. M. A micromethod for nonprotein nitrogen using two-tenths of a cubic centimeter of blood, 622
—. Sources of error in the Epstein method for blood sugar determination and a modified technic, 489
WILSON, ERIC R. (*See* Grant and Wilson), 562
WOOLLEY, PAUL G. Acne vulgaris, (*E*), 126
—. Basal metabolism, (*E*), 301
—. Carotinemia, (*E*), 436
—. Hypersensitiveness to foods as cause for abdominal pain, (*E*), 187
—. Intensive treatment and neurosyphilis, (*E*), 246
—. Sputum examination, (*E*), 308
WHYNN, JAMES. Effect of time between obtaining a spinal fluid and making a cell count on the result of the count, 273

Y

YOUNGBURG, GUY E. The use of open delivery tubes in the distillations when determining urea and nonprotein nitrogen in blood, 552
YARBROUGH, NANCY. Blood counts with oxalated blood, 172

SUBJECT INDEX

A

Abdominal pain, hypersensitive to foods as cause for, 187
Acetonitrile, effects of various foods, food factors and chemical agents upon resistance of animals to, 267
  tests, effects of thyroid, thyroxin and other iodine compounds upon, 349
Achylia gastrica, relation of to chronic focal infections and pernicious anemia, 452
Acidosis, chronic, chronic urticaria due to, 534
Acne Vulgaris, 126
Activities of the Medical Section of the League of Nations, 766
Agar, nutrient, changes in, due to clarifying with egg, 757
Air, expired, a spirometer for continuously and automatically measuring and sampling for analysis, 421
Albumin, effects of bile salts in urine on routine tests for, 743
Analysis of gastric content, devices to eliminate certain objectionable factors in, 431
Anemia, pernicious, relation of achylia gastrica to, 652
  therapeutic use of germanium dioxide in, 643
Animals, box for handling small, in laboratory, 761
Apparatus, laboratory, 477
Arsphenamine and neoarsphenamine dosage, 181
  constitution of, 127
  methyl alcohol in, 168
  some factors which influence its colloidal properties, 723
  sythesis of, 151, 215
Atmospheric moisture requirements of bacteria, 555
Atropine, differences in response to, in white and colored races, 104
  in blood serum, on determination of small quantities of, 280
Atrophy, muscular, nature and treatment of, 746
Auricle, flutter and fibrillation of, 242
Automatic apparatus for experiments with surviving organs, 751
Automatic pipetting device, 54

B

Baby respiration apparatus, sources of error in determining respiratory quotient in, 91
Bacteria and formed elements in the urine of rabbits, 589
  atmospheric moisture requirements of, 555
Bacteriology, studies in postmortem, 538
Bacteriophage, phenomenon of, 288
Bacterium diphtheriae, precipitin test in the detection of, 619
Basal metabolism, 301
    apparatus, mask for, 560
    determination and its technical difficulties, 191
      clinical value of, 669
    rate, gasometer method of the determination of, from carbon-dioxide elimination, 665
Bell and Doisy method of estimation of inorganic phosphorus in blood serum, 176
Benzyl compounds, pharmacology of, 69, 579
Beriberi: some facts and the possible hypothesis, 340
Bile salts in urine, effect of, on routine test for albumin, 743
Biliary fistula, technic for making, 84
Blood and serum, viscosimetry of, as aid in clinical diagnosis, 134
  chlorides in mercuric chloride nephrosis, 129
    in tungstic acid filtrates, 166
    counts with oxalated blood, 172
    determination of nitrogen in, 53
    flow in man as estimated by calorimetric method of Stewart, 439
    lactic acid in, determination of, 635
    pressure studies in one hundred-forty cases of diabetes mellitus, 392
    serum, determination of small quantities on atropine in, 280
      in cancer, refractoviscosimetric properties of, 660
      viscosimetric properties of, in tuberculosis, 733
    sugar content of capillary blood as compared with that of venous blood, 495
      determination, Epstein method for, sources of error, 489
      method, Folin and Hu, applicable to small amounts of blood, 357
    uric acid in, determination of, 551
Botulism, 116
Box for handling small animals in the laboratory, 761
Butter, elimination of molds from, 208

C

Caging and feeding mice, 299
Calorimetric method of Stewart for estimation of blood flow in man, 439
Cancer of the rectum, possible defensive factors in, 602
  refractoviscosimetric properties of the blood serum in, 660

Capillary blood, blood sugar content of, as compared with that of venous blood, 495
Carbon-dioxide elimination, gasometer method of the determination of basal metabolism rate from, 665
Carcinoma and carbohydrate metabolism, 294
Carbohydrate metabolism, carcinoma and, 294
Carotinemia, 435
Catalase, estimation of, 173
Cell count, effect of time between obtaining a spinal fluid and making, on result of count, 273
Cells, counting, in cerebrospinal fluid, 555
Cellular elements of the cerebrospinal fluid, 626
Centrifuge tube holder, 482
Cerebrospinal fluid, cellular elements of the, 626
colloidal benzoin reaction of, 400
counting the cells in, 555
isolation of tetanus bacilli from, 100
Cernes' method of Wassermann reaction, 15
Chair for performing spinal punctures, 430
Chemical control of respiratory center, 369
Chemotherapy of organic arsenicals and related physical phenomena, 17
Chronic nephritis with an unusual degree of nitrogen retention, 229
urticaria due to chronic acidosis, 534
Clinical consultant, laboratory specialist as, 410
diagnosis by the aid of viscosimetry of the blood and serum, 134
value of basal metabolism determination, 669
Cod liver oil in rickets, 571
Cold, common, ventilation, weather, and the, 39
Collodium sacs, making of, 564
Colloidal benzoin reaction of cerebrospinal fluid, 400
properties of arsphenamine, some factors with influence, 723
Color indices, syphilometric, 1
Complement-fixation reaction in the diagnosis of tuberculous meningitis, 427

D

Deficiency diseases, vitamins and, 60
Denervated muscle, treatment of, 433
Diabetes mellitus, blood pressure studies in, 392
Diabetic diet, 694
Diet, diabetic, 694
Digitalis, infusion of, experimental investigation of pharmacological activity of drug store samples of, 225
Diphtheria bacilli, differential staining of the granules in, 180
Dog, male, method of obtaining samples of urine from, 760
spinal, method of producing, 417

E

Elective localization of bacteria following various methods of inoculation, and the production of nephritis by devitalization and infection of teeth in dogs, 707
Enzyme poisoned by heavy metal salts, activation of an, 736
Eosinophilia in parasitic infection, 123
Epstein method for blood sugar determination, sources of error in, and a modified technic, 489
Etiology of rickets, 702
of scarlet fever, 592
Extracts, pancreatic, 464

F

Feeding and caging mice, 299
Fistula, biliary, technic of making, 84
Fistula, pancreatic, 86
Focal embolic glomerulonephritis, 507
infection, 573
infections, chronic, relation of achylia gastrica, to, 652
Folin and Hu blood sugar method applicable to small amounts of blood, 357
Foods, food factors and chemical agents, effects of various, upon the resistance of animals to acetonitrile, 267
Formaldehyde sterilizer, 484

G

Gasometer method of determination of the basal metabolism rate from carbon-dioxide elimination, 665
Gastric content, devices to eliminate certain objectionable factors in the analysis of, 431
German cure for tuberculosis, another, 120
Germanium dioxide in anemia, therapeutic use of, 643
Glomerulonephritis, focal embolic, 507
Glucose tolerance test, 293
Gonococcus, cultivation of the, 768

H

Heart disease, sociological aspects of, 606
Heavy metal salts, activation of an enzyme poisoned by, 736
Hematuria, role of hexamethylenamina in, production of, 514
Hexamethylenamina, role of, in production of hematuria, 514
Hypersensitiveness to foods as cause of abdominal pain, 187

I

Infection, focal, 573
Injection method for aiding in the identification of tapeworm species, 691
Intensive treatment of neurosyphilis, 246

Internal secretion, 763
of the pancreas, 251

K

Kjeldahl still, 57

L

Laboratory apparatus, new, 477
specialist as a clinical consultant, 410
technicians, a plea for the standardization of the training for, 562
Lactic acid in blood, determination of, 635
League of Nations, activities of Medical Section of, 766
Liver function, 565

M

Mailing case opener, 483
Mammary secretion, excess protein and, 505
Mask for basal metabolism apparatus, 560
Medical and health conditions in Soviet Russia, 248
fellowships, 502
needs of Russia, 436
Mercuric chloride nephrosis blood chlorides in, 129
poisoning, 233
Methyl alcohol in arsphenamine, 168
Mice, caging and feeding, 299
Microcolorimeter, 237
Micromethod for nonprotein nitrogen using two-tenths c.c. of blood, 622
Micromodification of the method of Benedict for the quantitative determination of reducing sugar in urine, 364
Microscopic examination, preparing tissues for, 114
Molds, elimination of, from butter, 208
Muscular atrophy, nature and treatment of, 746

N

National Board of Medical Examiners, 189
Nature and treatment of muscular atrophy, 746
Neoarsphenamine and arsphenamine dosage, 181
Nephritis, production of, by devitalization and infection of teeth in dogs, 707
Nervous control of the pyloric sphincter, 375
Neurosyphilis, intensive treatment and, 246
Nitrogen, in blood and urine apparatus for determination of, in, 53
retention, chronic nephritis with an unusual degree of, 229
Nonprotein and urea nitrogen, apparatus for determining simultaneously, 756
nitrogen, a micromethod for using two-tenths c.c. of blood, 622
Nonsyphilitic spinal fluids, 404
Nutrient agar, changes in, due to clarifying with egg, 757

O

Open delivery tubes in distillations when determining urea and nonprotein nitrogen in blood, 552
Organic arsenicals, chemotherapy of, 17
Oxalated blood, blood counts with, 172

P

Pancreas, internal secretion of, 251
Pancreatic extracts, 464
fistula, technic for establishment of, 86
Paraffin embedded material, time-saving devices for handling, 240
embedding and low temperature evaporation, removable water-bath tops for, 689
Parasitic infections, eosinophilia in, 123
Pathogenicity of B. botulinus, 570
Pellagra, further studies on, 631
Pharmacological action of quinidins, 311
Pharmacological activity of drug store samples of infusion of digitalis, 225
Pharmacology of Benzyl compounds, 69
further observations of, 579
Phenomenon of bacteriophage, 288
Phosphorus, inorganic, in blood plasma by the method of Bell and Doisy, 176
poisoning, treatment of acute, 148
Pipette dryer, 482
washer, 754
Pipetting device, automatic, 54
Poisoning, mercuric chloride, 233
phophorus, treatment of, 148
Postmortem bacteriology, 538
Precipitin test in the detection of bacterium diphtheriae, 619
Protein, excess, and mammary secretion, 505
Pyloric sphincter, nervous control of the, 375

Q

Quinidin, pharmacological action of, 311

R

Rack, test tube, 753
Rats, self-feeder for, 687
Recording spirometer, a new type of, 681
Rectum, cancer of the, possible defensive factors in, 602
Red cells, Rouleaux formation of, in various types of disease, 614
Refractoviscosimetric properties of the blood serum in cancer, 660
of blood serum in cases of tuberculosis, 733
Research fellowship in bacteriology, 503
Respiratory center, chemical control of, 369
illness, and school personnel, 44

Respiratory—Cont'd
quotient, sources of error in determining with baby, respiration apparatus, 91
Rickets, cod liver oil in, 571
etiology of, 702
Rouleaux formation of red cells in various types of disease, 614
Russia's medical needs, 436

S

Scarlet fever, etiology of, 592
Sealing bacterial cultures, influence of on spore formation, 199
Self-feeder for rats, 687
Serology of syphilis, 1
Shaking machine, 487
Shock, newer conceptions concerning, 498
Sociological aspects of heart disease, 606
Soviet Russia, medical and health conditions in, 248
Spinal cord, tuberculoma of, 96
dog, method for producing, 417
fluid cell count, 273
punctures, chair, for performing, 430
Spirometer for continually and automatically measuring expired air and sampling for analysis, 421
recording, 681
Spore-bearing anaerobes, separation of, from other spore-bearing bacteria, 692
formation, influence on, of sealing bacterial cultures, 199
Sputum examination, 308
Staining tray for smear preparations, 485
Sterilization of surgical instruments, 360
Sterilized cultures, Widal technic using, 547
Stewart's calorimetric method of estimation of blood flow in man, 439
Sugar in urine, a micromodification of the method of Benedict for the quantitative determination of reducing, 364
Surgical instruments, sterilization of, 360
Surviving organs, automatic apparatus for experiments with, 751
Synthesis of arsphenamine, 151, 215
Syphilimetric color indices, 1
Syphilis, serology of, 1

T

Tapeworm species, injection method for aiding in the identification of, 691
Teeth, infection and devitalization of, nephritis as result of, 707
Test tube rack, 753
Tetanus bacilli, insolation of, from cerebrospinal fluid, 100
Therapeutic use of germanium dioxide in anemia, 643
Thyroid, thyroxin and other iodine compounds, effects of, on acetonitrile, 349
Tissue, preparing for microscopic examination, 114
Toxicological effect of methyl alcohol, 170
Treatment of acute phosphorus poisoning, 148
Tuberculoma of the spinal cord, 96
Tuberculosis, another German cure for, 120
cure, new, 700
intimate exposure and contagion in, 306
refractoviscosimetric properties of the blood serum in cases of, 733
Tuberculous meningitis, complement-fixation reaction in the diagnosis of, 427
Tungstic acid filtrates, estimation of blood chlorides in, 166

U

Urea and nonprotein nitrogen, apparatus for determining simultaneously, 756
of blood, open delivery tubes in the distillations when determining, 552
concentration test, 233
Uric acid excretion, yeast therapy and, 473
Urine, determination of nitrogen in, 53
easy method of obtaining samples of, from male dog, 760
effect of bile salts in, on routine tests for albumin, 743
of rabbits, bacteria and formed elements in, 589
Urticaria, chronic, due to chronic acidosis, 534

V

Vacuum vaccine collector, 486
Venous blood, blood sugar content of capillary blood as compared with, 495
Ventilation, weather and the common cold, 39
Vitamin, effect of lack of, 268
Vitamins and deficiency diseases, 60
where do they come from, 504
Viscosimeter of Hess, 134
Viscosimetry of blood and serum, clinical diagnosis by the aid of, 134

W

Wassermann reactions, method of determining the number of complement-binding units in sera giving positive, 109
test tube and pipette washer, 754
Water-bath tops, removable, for paraffin embedding and low temperature evaporation, 689
Weather, ventilation and the common cold, 39
Widal Technic, for using sterilized cultures, 547
Woodhead, G. Sims, obituary, 705

Y

Yeast therapy and uric acid excretion, 473

6519